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DERRIS AS AN INSECTICIDE

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INTRODUCTION

The investigation of the possibilities of Derris as an insecticide is a continuation of the cooperative work inaugurated by the Bureaus of Entomology and Plant Industry, and the most important results pertaining to the study of Derris are discussed in this paper. There are now on the market several standard insecticides: Arsenicals, used as stomach poisons; nicotine solution, used as a contact insecticide; pyrethrum powder, employed as a dusting powder; and soaps, lime sulphur, oil sprays, etc. Not one of these acts both as a stomach poison and a contact insecticide. The following pages will show how well Derris acts in both of these ways.

In a search through the vegetable kingdom for plants possessing toxic principles with a view toward utilizing them as insecticide material, attention was directed to the large class of plants which are used extensively in the tropics as fish poisons. There are many hundreds of these plants, included in several families, and their habitat extends over practically the entire Tropics. That many of them belonging to particular families and genera display a remarkably toxicity to fish has long been known, and probably for ages the natives of the Tropics have used some of these plants as a means of catching fish.

While a plant toxic to fish need not necessarily be poisonous to insects, nevertheless, some of the fish poisons have already been recommended and used in the Orient as insecticides. If the fish poisons prove to be efficient insecticides, their practical utilization is at once suggested, because many of them are known to be very abundant in the Tropics. The present investigation deals with six or seven species, all belonging to the same genus, which is widely known as Derris.[2] Of these species only *Derris elliptica* Benth. seems to have been used widely as a means for catching fish; it is regarded as a powerful fish poison.

[1] A portion of the experimental part of this investigation was performed at the Insecticide Board's testing laboratory, located at Vienna, Va., by W. S. Abbott and E. W. Scott, Entomologist, Enforcement Insecticide Act, under the direction of the latter.

[2] Although this genus has commonly been known as Derris, the rules of botanical priority require the use of the name Deguelia of Aublet. Of the six species mentioned, the following have received names under Deguelia: *Deguelia elliptica* (Wall.) Taub. [*Derris elliptica* (Wall.) Benth.]; *Deguelia robusta* (Roxb.) Taub. [*Derris robusta* (Roxb.) Benth.]; *Deguelia timorensis* (DC.) Taub. [*Derris scandens* (Roxb.) Benth.] *Deguelia uliginosa* (DC.) Baill.; [*Derri. uliginosa* (DC.) Benth.]. S. F. BLAKE.

The material available for the present study was secured in most cases from various agricultural and botanical agencies through the Office of Foreign Seed and Plant Introduction, United States Department of Agriculture. The following is a list of the material used and the sources from which and through which it was secured: Powdered roots of a Derris species, most likely *Derris elliptica* Benth., from the open market where it is sold as an insecticide; roots of *D. elliptica*, called "tuba" or "toeba" in the Dutch East Indies, from the 's Lands Plantentuin, Buitenzorg, Java; stems of *D. uliginosa* Benth., from Mr. C. H. Knowles, Suava, Fiji Islands; stems of *D. koolgibberah*[1] Baill., and of *D. oligosperma*,[1] from the director of the Botanical Gardens at Brisbane, Queensland, Australia; roots of *D. scandens* Benth.; and stems and roots of *D. robusta* Benth., from the director of the Botanical Survey of Sibpur, Calcutta, India.

HISTORICAL REVIEW

The genus Derris, belonging to the family Papilionaceae, tribe Dalbergieae and subtribe Lonchoecarpinae, is practically native throughout the Tropics, but is far more abundant in the Old World than in tropical America. Its members are climbing shrubs, having trunks 3 or 4 feet in height and about 4 inches in diameter; the trunks send out numerous long branches, which climb over the neighboring vegetation, and the tips of which hang freely downward.

Watt (*10, p. 80*)[2] describes Derris as a genus of arborescent climbers or trees, and states that the roots of *Derris elliptica* furnish a useful insecticide for gardening purposes. A number of other species are mentioned in the literature as being used for fish poisons, and in some cases reference is made also to their use as insecticides; but these cited cases seem to be no better than mere reports. Correspondents in the Philippine Islands and Java report that *D. elliptica* is probably the species most commonly used as a fish poison. In all cases, so far as known, only the roots are employed. It seems that the most widespread treatment is one in which the roots are buried in mud, brackish mud preferred, for a period of several weeks; then the roots are crushed and placed in water inhabited by fish. The roots of Derris, in all probability *D. elliptica*, are used as insecticides in the Dutch East Indies; and a correspondent reports that Derris is commonly used by the Chinese gardeners in the Malay Peninsula as an insecticide and that the parts of the plant used are sold by Chinese storekeepers. However, it is said that the poison loses its activity when the plant is dried.

Hooker (*5, p. 43*) reports on a specimen of *Derris elliptica*, obtained from Singapore where it is known as "tubah" and where it is used as an insecticide; the roots are steeped in water and the resulting decoction is said to be an efficient insecticide for garden purposes.

[1] No record of the publication of these specific names could be found.

[2] Reference is made by number (italic) to "Literature cited," p. 200.

Probably the first investigator to report on a chemical examination of *Derris elliptica* was Greshoff (*3*) in 1890. He found the most important constituent of the bark on the root to be a nitrogen-free, non-glucocidal resin which he called "derrid." He describes this resin, which he did not succeed in obtaining in crystalline form, as readily soluble in alcohol, ether, chloroform, and amyl alcohol, but soluble with difficulty in water and potassium hydroxid. The yield obtained from the whole root was 2.5 to 3 per cent. The resin was found to be extremely toxic to fish.

Dymock, Warden, and Hooper (*2, p. 471*) record that in India *Derris uliginosa* is used as an insecticide against larvæ of insects.

In 1892 Wray (*11*) worked on *Derris elliptica* and appears to have been unaware of Greshoff's paper, because for the resinous principle which he isolated from the root in an impure state and which he used in his experiments on fish he proposed the name "tubain." This substance is without question the same as Greshoff's "derrid," judging from its physical properties. The crushed roots when boiled in a retort with water yielded an opalescent distillate, the odors of which strongly resembled those from the roots. This distillate was found poisonous to fish.

In 1899 van Sillevoldt (*9*), working on *Derris elliptica*, reported on the extraction of Greshoff's "derrid." He used practically the same method of extraction as did Greshoff and describes the "derrid" obtained as a yellow, amorphous powder. In the impure "derrid" he found a crystallizable substance which was very insoluble in ether, by which means it could be separated from the soluble portion of the "derrid." He found the melting point of "derrid" to be near 73° C. and he describes it as being readily soluble in alcohol, ether, benzol, aceton, glacial acetic acid, acetic ether, carbon disulphid, and chloroform, and very insoluble in petroleum, ether, and water. Van Sillevoldt assigned the formula $C_{33}H_{30}O_{10}$ to "derrid."

In 1902 Power (*8*) investigated the stems of *Derris uliginosa*. His results led to the conclusion that the poisonous constituent of the plant is a resin, thus concurring in the views of Greshoff and van Sillevoldt. He noted further that this resin consists of two components, one being soluble in chloroform and highly toxic to fish, and the other insoluble in chloroform and inactive to fish.

In 1911 van Hasselt (*4*) investigated the physiological action of "derrid" on fish, frogs, mice, rabbits, and cats, and studied its effects on the blood, respiration, circulation, intestinal tract, and nervous system. From his experiments he concluded that "derrid" is a powerful poison, causing characteristic symptoms in all the animals treated, and that it kills by causing respiratory paralysis.

In 1916 Campbell (*1*) investigated the poisonous actions of *Derris elliptica*, and his work seems to be the most recent along this line. He tested the water and saline extracts of the roots on fish, mosquito larvæ,

tadpoles, toads, and monkeys. The following are the salient conclusions of his investigation: (1) Boiling does not destroy the toxic action of the sap; (2) roots kept three months in a cupboard retain their strength; (3) milky extract introduced into a fish's stomach is rapidly fatal; (4) tadpoles are fatally affected, but stronger extracts are required to kill them than to kill fish; (5) much stronger doses are required to kill mosquito larvæ than to kill either fish or tadpoles; (6) the extract from 1/50 gm. of the roots when injected subcutaneously is fatal to toads, and the extract from 1/12 gm. causes death when introduced into the stomach; and (7) when the extract from 2 gm. of the roots is injected subcutaneously or introduced into a monkey's stomach death results. Campbell further states (*p. 134–135*):

> From the results on different animals it is evident that the poison affects the more highly developed members of the animal kingdom more readily than it does the primitive members. This is only to be expected since its action concerns the brain and one particular part of this, namely, the medulla oblongata.
>
> It could be used to destroy mosquito larvæ, but it should be used in solutions not weaker than 1 in 1,000, that is just enough of the extract should be added to the pool to make the water cloudy.

METHODS OF PREPARING AND TESTING EXTRACTS FROM DERRIS MATERIALS

The many preliminary experiments performed indicate that Derris (probably *D. elliptica*) is promising as a contact insecticide and as a stomach poison but is of no practical use as a fumigant. The best methods of applying it—whether in the form of powder, suspended in water or in the form of extract mixed with water or with soap solution—now remain to be determined.

A vegetable insecticide is usually applied either in the form of fine powder or as a spray mixture. This mixture may consist of any one of the following four combinations: (1) Powder suspended in water; (2) aqueous extract of the material diluted with water; (3) a solution consisting of water and a small amount of a concentrated form of the active constituent; and (4) a small quantity of a concentrated form of the active principle suspended in water.

Since Derris material must be imported, only dried roots and stems may be secured for insecticidal purposes. As already stated under the historical review, the natives pound the roots of Derris into a pulp which they then throw into the water inhabited by fish. This allows the juices of the plant to mix freely with the water and is the simplest way of obtaining a water extract, but will water remove the toxic principle after the roots have become dry? The chief object of the investigation discussed under the preceding heading was to make a study of the different methods of extracting Derris and to determine the value of various solvents in order that a simple and economical method might be devised for obtaining the active principle and applying the extracts.

QUANTITATIVE EXTRACTIONS OF DERRIS AND PRELIMINARY TESTS OF EXTRACTS OBTAINED

Five series of quantitative extractions were made as follows: In each series 20 gm. of fine powder of *Derris* sp. (probably *D. elliptica*) were exhausted successively with the following five solvents in the order named: First series, petroleum ether, ether, chloroform, alcohol, and water; second series, ether, chloroform, alcohol, water, and petroleum ether; third series, chloroform, alcohol, water, petroleum ether, and ether; fourth series, alcohol, water, petroleum ether, ether, and chloroform; and fifth series, water, petroleum ether, ether, chloroform, and alcohol. No heat was used in any of these extractions. Table I gives the percentages of extracts thus obtained. The sequence is shown by the letters a . . . a, b . . . b, c . . . c, etc., beginning with the first extraction in each case.

TABLE I.—*Successive quantitative extractions of Derris sp. with various solvents, starting with a different solvent for each series*

No. of extraction.	Solvents used.				
	Petroleum ether.	Ether.	Chloroform.	Alcohol.	Water.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
First	4.07 a	7.90 b	10.60 c	11.25 d	9.75 e
Second	4.55 e	4.90 a	.75 b	3.60 c	8.45 d
Third	.55 d	2.00 e	.20 a	3.30 b	5.00 c
Fourth	.50 c	.49 d	.50 e	2.59 a	5.05 b
Fifth	.10 b	.20 c	.20 d	1.10 e	10.80 a

Attention is called to the following points in the preceding table. From the first extractions it will be seen that petroleum ether is a poor solvent, while the other four may be called good ones; of these four, only alcohol and water can be regarded as economic solvents. Other points in this table will be referred to later. Since the amount of an extract need not necessarily correspond to its toxicity, the following preliminary tests were performed.

Experience has taught that the honeybee (*Apis mellifica* L.) is extremely sensitive to stomach poisons; therefore this insect was fed small quantities of the foregoing extracts in order to determine the degree of toxicity of each one. It was furthermore considered desirable to know the effect of heat on the extracts. Consequently five of these extracts were obtained without the application of heat and the other five with the use of it. The following method of procedure was employed: Since all of these extracts, except those obtained with water, have a consistency similar to that of thick paste and are not soluble in water, it was necessary to dissolve a small quantity of each in alcohol; therefore 0.4 gm. of the petroleum-ether extract was dissolved in 10 cc. of 95 per cent alcohol. The same method was employed for each one of the other nine extracts, including the water extract, so that the effect of the alcohol would be the same in all the tests; and then ¼ cc. of one of these solutions was mixed thoroughly with 5 cc. of honey in a small

feeder, which was so covered with wire that the bees could not waste any of the food. After the 10 feeders, containing supposedly poisoned food, had been placed in as many wire-screen cases, 50 normal bees were introduced into each case; the bees were thereafter observed carefully and the dead ones were counted at regular periods. As a control, honey containing the same amount of alcohol as mixed with the other food was used; and whenever the bees required more food, pure honey was given to them. These experiments were repeated and were so arranged that the probable errors were minimized. Reference to Table II (extracts No. 246–254) shows that all of these extracts, except the water extract, are almost equally toxic to the honeybee within 48 hours and that there is practically no difference in toxicity between the extracts obtained with the use of heat and without it. The water extract apparently had no effect on the bees tested. Similar results were obtained by using the same extracts against aphids, fall webworms (*Hyphantria cunea* Dru.), and tussock-moth caterpillars (*Hemerocampa leucostigma* S. and A.) (see No. 246–249, 252–253, Table IV, and No. 253, Table V). The water extract from the powder of *Derris* sp. (filtered mixtures) killed only a small percentage of the aphids sprayed (see lower half of Table IV), while the nonfiltered spray mixtures, consisting of powder and soap solution, were efficient against aphids.

To determine whether the solvents had removed all of the toxic principle from the powders extracted, these five powders (No. 240–244 in Table II) after having been thoroughly dried were fed to other honeybees in the same manner as already described. In these tests ⅛ gm. of powder was thoroughly mixed with 5 cc. of honey. Reference to Table II shows that the powders exhausted with ether, chloroform, and alcohol had very little effect on the bees tested, while the powder exhausted with water killed 94 per cent of the bees within 48 hours. The results pertaining to the powder exhausted with petroleum ether are not reliable (see note at bottom of Table II).

To ascertain the effect of powder exhausted successively with 1 to 4 of the solvents and also the effects of the resulting extracts, other experiments were performed. Reference to Table II (No. 260, 261, 264, and 266) shows that powder successively extracted is only slightly less effective than powder extracted once, and that the third and fourth successive extracts (No. 263 and 265) have no effect at all. These results agree in only certain respects with the successive quantitative extractions, expressed in Table I.

To determine whether any poisonous volatile substance can be removed from Derris by steam distillation, 50 gm. of the powder were so treated and the distillate was collected. Later some of this distillate and a portion of the distilled powder, after it had been dried, were tested on silkworms. The distillate had no effect whatever, but the powder was as poisonous as ever.

To facilitate the handling of a product which might be used as a proprietary insecticide, an alcoholic extract was incorporated into a soft linseed-oil soap at the rate of 1 gm. of extract to 4 gm of soap. This product was later dissolved in water in the proportion of 1 to 2,400, which would be equivalent to about 1 pound of powder to 200 gallons of soap solution. All of the small fall webworms sprayed with this solution died, but none of the controls sprayed with soap solution of the same strength died.

TABLE II.—*Effects on the honeybee of eating extracts and powders of Derris sp. (probably D. elliptica)*

No. of extract or powder used.	Extracts, powders, and controls.	Number of bees tested.	Percentage of bees dead within—		
			48 hours.	48 hours.	7 days.
			After eating extracts and controls.	After eating powders from which one or more extracts had been removed, and controls.	
246	Petroleum-ether extract (no heat used)	150	97	.....	.....
247	Petroleum-ether extract (heat used)	150	97	.....	.....
248	Ether extract (no heat used)	150	97	.....	.....
249	Ether extract (heat used)	150	96	.....	.....
250	Chloroform extract (no heat used)	150	93	.....	.....
251	Chloroform extract (heat used)	150	95	.....	.....
252	Alcoholic extract (no heat used)	150	96	.....	.....
253	Alcoholic extract (heat used)	150	95	.....	.....
254	Water extract (heat used)	150	5	.....	.....
	Control, alcohol in honey	150	8	.....	.....
	Control, honey alone	150	3	.....	.....
240	Powder exhausted with petroleum ether	100	.....	[a] 100	.....
241	Powder exhausted with ether	100	.....	4	.....
242	Powder exhausted with chloroform	100	.....	2	.....
243	Powder exhausted with alcohol	100	.....	6	.....
244	Powder exhausted with water	100	.....	94	.....
245	Powder not exhausted with any solvent	100	.....	98	.....
	Control, wheat flour in honey	100	.....	1	.....
258	Powder exhausted with petroleum ether	100	.....	[a] 98	[a] 100
260	Powder exhausted with above solvent and ether	100	.....	0	73
261	Powder exhausted with above solvents and chloroform	100	.....	1	16
264	Powder exhausted with above solvents and alcohol	100	.....	2	18
266	Powder exhausted with above solvents and water	100	.....	0	16
	Control, wheat flour in honey	100	.....	1	15
	Control, honey alone	100	.....	4	11
259	Petroleum-ether extract from original powder	100	67	.....	.....
262	Ether extract from above powder (2d extraction)	100	73	.....	.....
263	Alcoholic extract from above powder (3d extraction)	100	1	.....	.....
265	Water extract from above powder (4th extraction)	100	1	.....	.....
	Control, alcohol in honey	100	1	.....	.....

[a] This powder emitted an odor resembling that from petroleum ether; the bees ate very little of the honey containing it, and therefore most of them probably died for lack of suitable food.

Tables I and II show that 95 per cent ethyl alcohol is the only good economic solvent used and that heat has no effect on the extract obtained. It was decided, therefore, to make quantitative extractions of several species of Derris by using hot denatured alcohol, since this solvent is comparatively cheap.

TABLE III.—*Quantitative extractions of various species of Derris made with hot denatured alcohol*

Name of species.	Part of plant used.	Percentage of extract obtained.
Derris sp. (probably *D. elliptica*)	Roots	14.25
D. elliptica, called "tuba"	Roots	8.50
D. uliginosa	Stems	8.50
D. koolgibberah	Stems	10.30
D. scandens	Roots	20.30
D. oligosperma	Stems	22.50
D. robusta	Roots	16.70
D. robusta	Stems	15.70

The foregoing table shows that denatured alcohol is a good solvent and that the percentages of extract obtained vary considerably; this variation is certainly due in part to the fact that the eight powders used varied considerably in fineness. Results showing the effectiveness of these extracts are discussed on page 188 and in Table V.

EXTRACTION OF TOXIC PRINCIPLE FROM DERRIS SP. BY TWO METHODS

As already stated, Greshoff (*3*), van Sillevoldt (*9*), and Powers (*8*) have agreed that the toxic principle in *Derris elliptica* and. *D uliginosa* is a resin and have called the active portion of it "derrid." In the present investigation it was considered expedient to isolate a small quantity of the resin and to test it on insects and on a few higher animals.

VAN SILLEVOLDT'S METHOD

One kilo of the powdered root of *Derris* sp. was repeatedly extracted with boiling water until the extract was only slightly colored. After the powder had been filtered and thoroughly dried it was boiled under a reflux condenser with successive portions of 95 per cent alcohol until exhausted. The combined alcoholic extracts were mixed with one-fourth their volume. of water, and the alcohol was distilled under reduced pressure. As the alcohol was removed, the material in the flask became milky in appearance and the resinous substance collected in a mass on the bottom of the flask The last portion of the water was removed by transferring the material to an open dish on a steam bath. The residue was a resinous, sticky mass which weighed 110 gm., representing 11 per cent of the dry root. It was

dissolved in boiling alcohol, and then the solution was heated with animal charcoal and filtered. Upon evaporation, the resin closely resembled the appearance it had before being treated with the charcoal and seemed to consist of two forms, the greater portion being of a soft and pliable nature, while the other portion was hard and brittle. The latter had a melting point of 66°–68° C. Van Sillevoldt reports the melting point of "derrid" as being about 73° C.

Two gm. of the soft portion were dissolved in 50 cc. of 95 per cent alcohol by means of a low heat; upon standing, a fine, yellowish-white powder settled to the bottom of the flask; then this powder was separated by means of a force filter, and after being washed with a small quantity of alcohol and ether it was dried. This material appeared like an amorphous powder, but under the microscope it was found to consist of small plate-like crystals. The melting point of these crystals was 170° C.

A dilute alcoholic solution of the above crystals, as well as the alcoholic solution of the resin from which the crystals had been separated, was found to be very toxic to fish. A subcutaneous injection of 0.00066 gm. of the crystals was fatal to a mouse in two hours.

The preceding method of extracting the resin is not very practicable on a large scale. Several of the operations involved could possibly be dispensed with.

POWER'S METHOD

One kilo of the powdered root of *Derris* sp. was repeatedly extracted with boiling alcohol until exhausted. Upon removal of the alcohol by distillation under reduced pressure, 173 gm. of a dark extract of a pillular consistency were obtained; this amount is equivalent to 17.3 per cent of the dry root. Then the extract was repeatedly extracted under a reflux condenser with hot petroleum ether until the latter was no longer colored. From the combined extracts the petroleum ether was removed and a waxy, yellow residue weighing 16 gm. remained. This residue was designated *A*.

The alcoholic extract after having been exhausted with petroleum ether was heated on a steam bath with 95 per cent alcohol until it was brought into solution, whereupon it was poured slowly into a large quantity of cold water; a fine suspended precipitate resulted. The precipitated resin was filtered by means of a force filter, then washed with water, dried by means of an electric fan, and finally pulverized to a No. 20 powder which was grayish in color and weighed 102 gm., being equivalent to 10.2 per cent of the original material. This was designated *B*.

Sixty-five gm. of the resin *B* were extracted in a Soxhlet extractor with chloroform until exhausted; 11.4 gm. or 17.5 per cent remained undissolved. This portion was removed from the extractor, was dissolved in alcohol, and then precipitated in cold water. After the precipitate had

been filtered and dried, a chocolate-brown powder resulted. This was designated *C*.

The chloroform extract from *B* was placed on a steam bath to remove the chloroform. The residue resulting was a dark, sticky material which became hard and brittle when cooled below room temperature. It was ground while hard and was designated *D*.

The three substances designated *A*, *C*, and *D* were tested in very dilute form on small chinook salmon and were found to be exceedingly toxic. The extract *A* appeared to be the most powerful, while the chloroform-soluble resin *D* was much more toxic than was *C*. The effect of the extract *A* on the fish might have been influenced to a considerable extent by a trace of petroleum ether which seemed to remain in the extract and imparted to it a distinct odor.

The three substances called *A*, *C*, and *D* were tested also on small tent caterpillars by being sprayed on foliage. Within eight days *A* had killed 70 per cent, *C* 92.3 per cent, and *D* 54.4 per cent of the caterpillars tested; but only 22.1 per cent of the control larvæ had died.

EXTRACTION OF DERRIS ELLIPTICA AND TESTS OF EXTRACTS OBTAINED

The roots of "tuba" or "toeba" were ground as fine as their fibrous nature would permit, and 200 gm. of this powder were macerated for two days with a quantity of cold water. After the mixture had been filtered, the water extract measured 600 cc., each cc. representing ⅓ gm. of the roots. Half of this cold water extract was tested on small tent caterpillars; within eight days only 30.9 per cent of them had died. The other half of this extract was evaporated to one-half its volume on a steam bath and then again made up to its original volume with water. This portion of the extract was later tested on small tent caterpillars; within eight days only 14.3 per cent of them had died. This does not mean that the application of heat affected the toxicity of the extract, for 22.1 per cent of the control larvæ died.

The marc from the preceding water extractions was dried by means of a current of air and was macerated with several portions of cold petroleum ether. The combined extracts were then divided into two equal portions. While the petroleum ether evaporated spontaneously from one portion in an open dish, it evaporated on a steam bath from the other portion. The residue resulting was a waxy, yellow substance which represented 1.4 per cent of the original material. Spray solutions containing these petroleum-ether extracts were tested on aphids; there was practically no difference in effectiveness between the extract obtained without the use of heat and the one with it (see No. 288 and 289, Table IV).

The powder left after the preceding extractions was spread out, and the residual petroleum ether was allowed to evaporate. It was then

divided into two equal parts; one part was macerated with successive portions of cold 95 per cent alcohol until exhausted, and the other part was boiled on a steam bath with successive portions of 95 per cent alcohol until exhausted. The combined extracts from the first part represented 4.17 per cent of the original powder and those from the second part 4.26 per cent. Spray solutions containing these alcoholic extracts were tested on aphids, small fall webworms, and on large tussock-moth caterpillars. There was practically no difference in their effectiveness on these insects (see No. 290 and 291, Table IV).

EXTRACTION OF DERRIS ULIGINOSA AND TESTS OF EXTRACTS OBTAINED

The stems were reduced to a coarse powder, and 100 gm. of this material were repeatedly extracted on a steam bath with petroleum ether until exhausted. Upon evaporation of the petroleum ether, there remained a yellow, shiny, somewhat brittle substance which represented 1.02 per cent of the original stems.

The marc from the above extraction was dried thoroughly and then exhausted with 95 per cent alcohol on a steam bath. The residue left upon the evaporation of the alcohol represented 7.82 per cent of the stems. The above petroleum-ether and alcoholic extracts were found very effective against aphids (see No. 293 and 294, Table IV).

EXTRACTION OF VARIOUS SPECIES OF DERRIS WITH DENATURED ALCOHOL AND TESTS OF EXTRACTS OBTAINED

Since the preceding results have shown that alcohol is the most suitable solvent for the toxic resins, the use of denatured alcohol as the best economic solvent was at once suggested. By the use of suitable apparatus this solvent can be recovered with very little loss and consequently can be used repeatedly.

For the tests described below, 50 gm. of powdered material in each instance were extracted with denatured alcohol on a steam bath, and the extract was concentrated to 25 cc. so that 1 cc. was equivalent to 2 gm. of material.

In the tests performed in the laboratory, the general plan for each test was to spray or dust about 500 aphids or 100 caterpillers on foliage, and then to place this foliage in a bottle of water inside a battery jar which was covered with cheesecloth. A record of the dead insects was taken at regular periods. The tests with aphids usually covered a period of 24 hours, and those with caterpillars and potato beetles (*Leptinotarsa decemlineata* Say) 10 or 12 days. The results of most of these tests are given in Tables IV and V. Table V gives chiefly the results obtained by using denatured alcoholic extracts and the powders of various species of Derris, applied as dust. Attention is called to the

following points: (1) The alcoholic extracts of *elliptica*, *uliginosa*, and *koolgibberah* (No. 296, 295, and 298) were generally efficient, while those of *oligosperma*, *scandens*, and *robusta* (No. 299, 300, 400, and 401) were only seldom efficient; (2) the powder of *Derris* sp. (No. 110), mixed with water or soap solution, was usually efficient, while the other powders (No. 402–406) tested by this method were found inefficient; and (3) of the eight powders used as dusts, only those of *Derris* sp., *elliptica*, and *uliginosa* (No. 110, 408, and 407) were found efficient.

TABLE IV.—*Effects of Derris extracts as contact insecticides and stomach poisons, comparing the results of the extracts derived with the aid of heat and without heat, and those dissolved in water and in soap solution*

Species of Derris used.	Kind of extracts and controls.	No. of the extracts used.	Temperature conditions during the extraction.	Percentage of aphids and caterpillars killed, ratio of extract to solvent, and kind and strength of solvents.										
				Myzus persicae Sulz.		*Macrosiphum liriodendri* Mon.					Fall webworm.		Tussock-moth caterpillar.	
				[a] 1:100	[b] 1:100	[a] 1:100	[c] 1:100	[d] 1:200	[e] 1:300	[f] 1:300	[g] 2:100	[h] 2:100	[g] 2:100	[h] 2:100
Derris sp.	Petroleum-ether extract	246	No heat used	19.6	53.2	.....	83.3	.....	.....	.....	.....	.....	.....	.....
		247	Heat used	27.0	59.1	.....	93.1	.....	.....	.....	.....	.....	.....	.....
	Ether extract	248	No heat used	64.8	92.5	.....	98.3	.....	.....	.....	.....	.....	.....	.....
		249	Heat used	54.6	90.9	.....	95.3	.....	.....	.....	.....	.....	.....	.....
	Alcoholic extract	252	No heat used	33.3	93.5	.....	95.4	73.4	92.1	100.0	65.0	44.0	66.6	60.0
		253	Heat used	62.2	75.7	.....	93.7	51.7	96.4	98.8	75.3	40.0	67.3	42.2
D. elliptica	Alcoholic extract	[i] 290	No heat used	.....	.....	72.8	98.7	86.4	99.4	100.0	81.7	73.1	60.0	88.2
		[i] 291	Heat used	.....	.....	73.1	97.3	70.0	96.5	100.0	77.7	64.4	60.0	76.1
	Petroleum-ether extract	[j] 288	No heat used	.....	.....	53.7	86.2	.....	.....	.....	.....	.....	.....	.....
		[j] 289	Heat used	.....	.....	68.8	87.7	.....	.....	.....	.....	.....	.....	.....
D. uliginosa	Petroleum-ether extract	[i] 293	Heat used	.....	.....	.....	.....	48.1	81.9	95.5	.....	.....	.....	.....
	Alcoholic extract	[i] 294	Heat used	.....	.....	.....	.....	38.2	96.1	97.4	.....	.....	.....	.....
Controls	Alcohol in soap solution	.....	.....	.....	11.6	.....	24.2	11.1	14.5	90.1	.....	15.2	.....	54.0
	Soap solution only	.....	.....	.....	.....	.....	15.9	7.0	16.7	88.2	.....	22.2	.....	25.0
	Water only	.....	.....	.....	.....	4.7	.....	9.2	4.0	1.9	31.1	.....	.....	.....
	Soaked in water five days (filtered)	.....	.....	.....	.....	.....	57.5	.....	.....	.....	.....	.....	.....	.....
	Soaked in water five days (not filtered)	.....	.....	.....	.....	.....	98.6	.....	.....	.....	.....	[k] 35.3	.....	.....
	Soaked in water two days (filtered)	.....	.....	.....	.....	[i] 31.4	.....	.....	.....	.....	.....	.....	.....	.....
D. sp.	Soaked in water two days (not filtered)	.....	.....	.....	.....	.....	.....	.....	[m] 100.0	.....	52.0	.....	.....	.....
	Soaked in soap solution two days (filtered)	.....	.....	.....	.....	.....	46.0	.....	.....	.....	.....	.....	.....	.....
	Soaked in soap solution two days (not filtered)	.....	.....	.....	.....	.....	96.5	.....	.....	.....	.....	.....	.....	.....
	Proprietary extract 1:400[k]	.....	.....	.....	.....	.....	.....	90.9	100.0	87.4	.....	.....	.....	.....

[a] Ratio of extract from 1 pound of powder to 100 gallons of water.
[b] Ratio of extract from 1 pound of powder to 100 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[c] Ratio of extract from 1 pound of powder to 100 gallons of soap solution. Soap solution made by adding 1 pound of fish-oil soap to 100 gallons of water.
[d] Ratio of extract from 1 pound of powder to 200 gallons of soap solution. Soap solution made by adding 1 pound of fish-oil soap to 100 gallons of water.
[e] Ratio of extract from 1 pound of powder to 200 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[f] Ratio of extract from 1 pound powder to 200 gallons of soap solution. Soap solution made by adding 4 pounds of fish-oil soap to 100 gallons of water.
[g] Ratio of extract from 2 pounds of powder to 100 gallons of water.
[h] Ratio of extract from 2 pounds of powder to 100 gallons of soap solution. Soap solution made by adding 1 pound of fish-oil soap to 100 gallons of water.
[i] See p. 187.
[j] See p. 186.
[k] Ratio of extract to water 1:100, soap being added later at rate of 4:100.
[l] Ratio of extract to water 2:100.
[m] Ratio of extract to water 2:100, soap being added later.

TABLE V.—*Effects of alcoholic extracts and powders of various species of Derris as contact insecticides and stomach poisons, obtained inside and outside the laboratory*

Extracts and powders used.	Species of Derris used.	Kind of extracts and powders used and controls.	No. of the extracts and powders used.	Percentage of aphids and potato beetles killed, ratio of extract to solvent, and kind and strength of solvent.										
				Illinoia sp.		*Macrosiphum liriodendri* Mon.	*Aphis helianthi* Mon.	*Rhopalosiphum pseudobrassicae* Davis.		*Aphis gossypii* Glover.	*Aphis spiraecola* Patch.	Colorado potato beetles.		
												Outside laboratory.		In potato patch.
				Inside laboratory.	Outside laboratory.	Inside laboratory.	Inside laboratory.	In turnip patch.	Inside laboratory.	Inside laboratory.	Outside laboratory.	Larvæ.	Adults.	Larvæ.
				[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[b] 1:100	[c] 1:50	[c] 1:50	[c] 1:50
	Derris sp.	Alcoholic extract	253	.....	.....	96.4	78.7	.....	.....	90.0	.....	.....	.....	.....
	D. elliptica	Alcoholic extract	[d] 291	.....	.....	96.5	86.1	.....	.....	96.0	.....	.....	.....	.....
		Denatured alcoholic extract	296	.....	.....	.....	94.8	98.0	100.0	100.0	30.0	96.6	29.1	.....
	D. uliginosa	Denatured alcoholic extract	295	.....	.....	.....	92.0	95.0	100.0	100.0	30.0	98.5	25.0	.....
		Alcoholic extract	[e] 294	.....	.....	96.1	.....	.....	.....	.....	.....	.....	.....	.....
	D. koolgibberah	Denatured alcoholic extract	298	.....	.....	.....	94.6	.....	100.0	100.0	30.0	.....	.....	.....
	D. oligosperma	do	299	.....	.....	.....	54.0	.....	60.0	65.0	20.0	.....	.....	.....
	D. scandens (roots)	do	300	.....	.....	.....	78.0	.....	95.0	82.0	30.0	.....	.....	.....
	D. robusta (roots)	do	400	.....	.....	.....	23.7	.....	99.0	50.0	30.0	.....	.....	.....
	D. robusta (bark)	do	401	.....	.....	.....	47.4	.....	99.0	60.0	30.0	.....	.....	.....
	Control	Soap solution only	.....	23.3	15.0	14.5	14.2	.....	20.0	25.0	20.0	.....	.....	.....

[a] Ratio of extract from 1 pound of powder to 200 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[b] Ratio of extract from 1 pound of powder to 100 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[c] Ratio of extract from 1 pound of powder to 50 gallons of water.
[d] Ratio of extract from 2 pounds of powder to 100 gallons of soap solution. Soap solution made by adding 1 pound of fish-oil soap to 100 gallons of water.
[e] Ratio of extract to water 1:100, soap being added later at rate of 4:100.

TABLE V.—*Effects of alcoholic extracts and powders of various species of Derris as contact insecticides and stomach poisons, obtained inside and outside the laboratory*—Continued.

Extracts and powders used.	Species of Derris used.	Kind of extracts and powders used and controls.	No. of the extracts and powders used.	Percentage of aphids and potato beetles killed, ratio of extract to solvent, and kind and strength of solvent.										
				Illinoia sp.		*Macrosiphum liriodendri* Mon.	*Aphis helianthi* Mon.	*Rhopalosiphum pseudobrassicae* Davis.		*Aphis gossypii* Glover.	*Aphis spiraecola* Patch.	Colorado potato beetle.		
												Outside laboratory.		In potato patch.
				Inside laboratory.	Outside laboratory.	Inside laboratory.	Inside laboratory.	In turnip patch.	Inside laboratory.	Inside laboratory.	Outside laboratory.	Larvæ.	Adults.	Larvæ.
				[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[a] 1:200	[b] 1:100	[c] 1:50	[c] 1:50	[c] 1:50
Extracts and powders in solutions, sprayed upon insects and plants.	*D.* sp. (probably *D. elliptica*).	Powder soaked in water ½ hour.	110	.....	.....	.....	.....	.....	.....	.....	.....	100.0	70.0	100.0
		Powder soaked in soap solution ½ hour.	110	100.0	90.0	.....	96.6	.....	.....	100.0	.....	.....	.....	.....
		Powder soaked in soap solution 2 days.	110	100.0	.....	.....	.....	.....	.....	.....	50.0	.....	.....	.....
	D. koolgibberah (stems)	Powder soaked in soap solution ½ hour.	402	75.8	50.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
	D. oligosperma (stems)	do	403	66.7	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
	D. scandens (roots)	do	404	60.0	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
	D. robusta (roots)	do	405	90.6	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
	D. robusta (bark)	do	406	70.3	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
	D. sp. (roots)	Dusted	110	97.7	.....	92.3	88.6	.....	100.0	99.0	.....	.....	.....	.....
	D. koolgibberah (stems)	do	402	75.9	.....	.....	10.0	.....	95.6	50.0	.....	.....	.....	.....
	D. oligosperma (stems)	do	403	34.9	.....	.....	9.7	.....	87.5	10.0	.....	.....	.....	.....
Powders dusted upon insects and plants.	*D. scandens* (roots)	do	404	34.1	.....	.....	4.0	.....	90.6	4.5	.....	.....	.....	.....
	D. robusta (roots)	do	405	33.3	.....	.....	3.0	.....	91.0	5.0	.....	.....	.....	.....
	D. robusta (stems)	do	406	13.2	.....	.....	16.8	.....	98.3	5.0	.....	.....	.....	.....
	D. uliginosa (stems)	do	407	.....	.....	.....	.....	.....	96.0	.....	.....	.....	.....	.....
	D. elliptica (roots)	do	408	.....	.....	.....	.....	.....	100.0	.....	.....	.....	.....	.....
	Control	Wheat flour	.....	2.5	.....	.....	4.1	.....	5.8	2.0	.....	.....	.....	.....

[a] Ratio of extract from 1 pound of powder to 200 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[b] Ratio of extract from 1 pound of powder to 100 gallons of soap solution. Soap solution made by adding 2 pounds of fish-oil soap to 100 gallons of water.
[c] Ratio of extract from 1 pound of powder to 50 gallons of soap solution.

EFFICIENCY OF DERRIS AS AN INSECTICIDE

The experiments relating to the efficiency of Derris as an insecticide were performed at the Insecticide Board's Testing Laboratory, located at Vienna, Va. The Derris material used was purchased on the open market and is distinguished from the other materials employed by being called powder derived from *Derris* sp.

EFFICIENCY OF DERRIS AS A CONTACT INSECTICIDE

The commercial powder, when used as a contact insecticide, was applied in two forms: (a) as a dry powder and (b) as a spray mixture with or without soap.

DERRIS APPLIED AS A POWDER AGAINST VARIOUS INSECTS

DOG FLEAS.—Eight dogs badly infested with fleas (*Ctenocephalus canis* Curt.) were dusted thoroughly. The material was applied with a shaker and well rubbed into the hair with the hands. At the end of 48 hours no living fleas were observed. Several dead ones were seen still clinging to the hairs.

CHICKEN LICE.—Twelve hens badly infested with several species of lice (Mallophaga) were thoroughly treated with the powder, which was well rubbed in through the feathers. When the hens were examined two or three days later, they were free from lice.

CHICKEN MITES.—When this powder was freely dusted over the chicken mites (*Dermanyssus gallinae* Redi), confined in jars, all were killed within 24 hours, but when used under practical conditions in a badly infested chicken house, all of the mites were not killed.

BEDBUGS.—Derris was tested against bedbugs (*Cimex lectularius* L.) by placing 20 bugs in a jar with a quantity of excelsior and then thoroughly dusting the contents of the jar. In nine tests under these very severe conditions 24.4 per cent of the bugs were killed in 24 hours and 52.8 per cent in four days. This material would be of no practical value against bedbugs.

ROACHES.—Six small cages were thoroughly dusted and 20 roaches (*Blattella germanica* L.) were placed in each cage. At the end of one week an average of 57.5 per cent of the roaches were dead, which indicates that this material would be of very little value under practical conditions.

HOUSE FLIES.—In cage tests, where house flies (*Musca domestica* L.) were dusted in ordinary flytraps about 10 inches high, all were dead or inactive within 24 hours. In room tests, where the powder was freely blown into the air and all parts of the room with a small hand dust gun, all of the flies were dead at the end of 16 hours.

In one test several hundred flies were liberated in a room which had been thoroughly dusted seven days before. Twenty-four hours later very few active flies were to be seen, and on the second day only three or four were living.

PLANT INSECTS.—Derris applied as a dust was of no value against the mealy bug (*Pseudococcus citri* Risso), the Orthezia (*Orthezia insignis* Doug.), red spiders (*Tetranychus bimaculatus* Harv.), and the crawling young of the oyster-shell scale (*Lepidosaphes ulmi* L.); but it was effective against nasturtium aphids (*Aphis rumicis* L.) and the green apple aphis (*Aphis pomi* De Geer).

DERRIS APPLIED AS A SPRAY MIXTURE

Derris applied as a spray mixture was tested against the green apple aphis (*Aphis pomi* De Geer) under field conditions and was found to be very effective. Young apple trees, about 10 feet high, were used. The spray mixtures were applied with a knapsack sprayer, except in tests No. 3 and 6, in which a barrel sprayer was used. When soap was used it was employed at the rate of 1 pound to 25 gallons of water. One dusting experiment was performed, the powder being applied with a large hand duster.

TABLE VI.—*Results of field tests, using Derris powder in spray mixtures and as a dust against the green apple aphi (Aphis pomi De Geer)*

No. of test.	Ratio of powder to water or soap solution used, dusting test, and controls.	Number of trees used.	Duration of tests.	Aphids killed.
			Days.	*Per cent.*
1	1 pound of powder to 25 gallons of water	5	3	100
2	1 pound of powder to 50 gallons of water	5	3	100
3	.....do	32	4	95–100
4	1 pound of powder to 50 gallons of soap solution	2	3	100
5	1 pound of powder to 100 gallons of water	3	3	100
6	.....do	52	4	80–90
7	1 pound of powder to 100 gallons of soap solution	5	3	100
8	1 pound of powder to 150 gallons of water	5	3	100
9	1 pound of powder to 150 gallons of soap solution	5	3	100
10	1 pound of powder to 200 gallons of water	5	4	[a] 98–100
11	1 pound of powder to 200 gallons of soap solution	5	3	[a] 98–100
12	Dusting test	2	2	100
13	Control, soap solution only	4	4	0
14	Control, trees untreated	8	4	0

[a] Four trees entirely free of aphids.

Table VI shows that Derris, even at the rate of 1 pound to 200 gallons of water, was very effective against the green apple aphis under field conditions and that on apple foliage the addition of soap does not increase its effectiveness. It also shows that this powder is effective as a dust.

Under greenhouse conditions, in tests against the nasturtium aphis, this material was found to be effective when used at the rate of 1 pound

of powder to 400 gallons of water, with soap at the rate of 1 pound to 100 gallons.

OYSTER-SHELL SCALE.—At the rate of 1 pound of powder to 20 gallons of water, either with or without soap in the proportion of 1 pound to 100 gallons, Derris was ineffective against the crawling young of the oyster-shell scale (*Lepidosaphes ulmi* L.).

While taking records of numerous greenhouse tests with Derris against aphids, it was noticed that all the aphids were not killed during the first 24 hours but continued to die for several days. Since a contact insecticide which continued to kill for a period of five or six days seemed an anomaly, the following experiments were made to determine definitely if this were the case and over how long a period this killing would extend.

The aphids on small potted plants, were counted, and the plants were then thoroughly dusted or sprayed. Paper disks were placed around the plants to catch the aphids that fell. Careful counts were made every day until all of the aphids were gone. In these counts each aphid was observed through a lens, and when necessary each one was touched with the point of a knife to determine whether it was still alive. A single untreated plant was used with each series as a control.

The aphids began falling from the plants within an hour, but for the first 24 hours most of those on the paper disks were alive. After this the aphids that fell were practically all dead. In the case of the dusted plants a few dead aphids were found clinging to the leaves the third day, but as a rule only the living ones remained on the plants.

These tests fully confirmed the earlier observations and, furthermore, showed that some of the aphids did not die until five or six days after the application of the insecticide. The results are presented in Tables VII and VIII.

TABLE VII.—*Results of tests against nasturtium aphids (Aphis rumicis L.), using Derris powder in spray mixtures at the rate of 1 pound of powder to 100 gallons of water*

Number.	Percentage of aphids living on plant at end of—						
	First day.	Second day.	Third day.	Fifth day.	Sixth day.	Seventh day.	Eighth day.
Aphids treated:							
182	52.2	24.7	10.4	4.4	2.2	0.5	0.5
150	25.3	22.6	7.3	2.0	2.0	.0	.0
209	19.1	11.0	9.0	8.1	5.7	3.3	2.8
172	33.7	20.3	23.2	19.7	19.7	18.6	12.7
Average	35.0	19.6	12.7	8.5	7.4	5.6	4.0
Aphids untreated:							
159	96.2	105.6	104.4	137.1	144.6	169.7	235.2

TABLE VIII.—*Results of tests against aphids (Myzus persicae Sulz.) on cabbage plants, using Derris powder as a dust*

Number.	Percentage of aphids living on plant at end of—								
	First day.	Second day.	Third day.	Fourth day.	Sixth day.	Eighth day.	Tenth day.	Thirteenth day.	Fifteenth day.
Aphids treated:									
96	43.7	36.4	31.2	16.6	3.1	0.0	0.0	0.0	0.0
157	38.4	21.0	6.3	2.5	.6	.0	.0	.0	.0
111	49.5	29.7	11.7	9.0	4.5	1.8	.9	.9	.0
235	47.6	31.0	16.5	15.3	9.7	2.1	1.2	.4	.0
Average	44.8	29.5	16.4	10.8	4.5	1.0	0.5	0.3	0.0
Aphids untreated:									
180	103.3	128.3	146.1	179.4	231.6	315.5	315.5+	315.5+	315.5+

Reference to Tables VII and VIII shows that the percentage of untreated aphids gradually increased from the first day of the tests onward; This increase was due to the birth of aphids on the untreated plants. aphids were born likewise on the treated plants from the time the insecticide was applied until all the reproducing females had died. Since practically all of the aphids on the treated plants were dead at the close of the tests, the newly born young ones must have been killed by coming in close proximity to the particles of powder still remaining on the plants.

EFFICIENCY OF DERRIS AS A STOMACH POISON AGAINST VARIOUS INSECTS

POTATO-BEETLE LARVÆ.—Derris powder as a stomach poison was tested on a small scale against potato-beetle larvæ (*Leptinotarsa decemlineata* Say) at several strengths, ranging from 1 pound of powder to 16 gallons of water up to 1 pound to 128 gallons and was found to be very effective. Practically all of the larvæ were killed within 48 hours and the plants were little eaten.

Since these spray mixtures might have acted as contact poisons, because the larvæ were already on the plants when the latter were sprayed, a second series of tests was arranged to eliminate this factor. The same plants were used and from 20 to 40 larvæ were placed on them one or two days after they had been sprayed. The results obtained were practically the same as in the first series of tests. Very few living larvæ were found three days later and the plants were little eaten.

When applied as a dust, Derris was equally efficient against potato-beetle larvæ.

TENT CATERPILLARS.—Derris was tested against young tent caterpillars (*Malacosoma americana* Fab.) in a series of strengths ranging from 1 pound of powder to 8 gallons of water to 1 pound to 200 gallons. All the mixtures were found to be effective.

Apple tree branches were thoroughly sprayed, and after the foliage had dried from 20 to 40 newly hatched larvæ were placed on each branch. The caterpillars began to show signs of discomfort within 48 hours and were practically all dead in from 5 to 10 days. In no case was any material amount of feeding observed.

In a second series of tests the larvæ were placed on the branches and sprayed after they had begun to form their tents. Under these conditions sprays containing 1 pound of powder to 50 gallons of water and 1 pound to 100 gallons killed all of the larvæ within 24 hours. When 1 pound to 200 gallons and 1 pound to 400 gallons were used all the larvæ were not killed within 11 days, but the few which remained alive were very small and inactive.

Used as a dust, this material killed all of the treated larvæ within one week.

FALL WEBWORMS.—These caterpillars (*Hyphantria cunea* Dru.), about one-third grown, were killed within a week by a spray containing 1 pound of powder to 5 gallons of water. Mixtures ranging from 1 pound to 50 gallons to 1 pound to 200 gallons were not satisfactorily effective, since nearly all of the sprayed foliage was eaten and not all of the caterpillars were killed.

OAK WORMS.—Two small oak trees, on which about 300 caterpillars (*Anisota senatoria* S. and A.) were feeding, were sprayed thoroughly with Derris at the rate of 1 pound of powder to 25 gallons of water; soap was added at the rate of 1 pound to 50 gallons, and a knapsack sprayer was used. Within 24 hours the larvæ became inactive and ceased to feed, and at the end of 6 days no living ones could be found. As a check on this test, powdered arsenate of lead was applied at the rate of 1 pound to 50 gallons of water, and almost identical results were obtained.

A second test was made in which a small tree was sprayed, and 24 hours later about 50 larvæ were placed on it. The caterpillars ate very little and gradually disappeared, evidently leaving the tree, since no dead ones were observed; and at the end of 5 days they were nearly all gone.

DATANA LARVÆ.—Two apple trees, on which large colonies of nearly full grown apple datanas (*Datana ministra* Dru.) were feeding, were sprayed with Derris at the rate of 1 pound of powder to 50 gallons of water. Twenty-four hours later one living larva was found on one tree and two on the other. The ground under the trees was thickly sprinkled with dead larvæ and many had lodged in the trees.

CABBAGE WORMS.—In two cage tests against cabbage loopers (*Autographa brassicae* Riley), Derris, applied at the rate of 1 pound to 25 gallons of water, killed all of the larvæ within 24 hours.

PHARMACOLOGICAL EFFECTS OF TOXIC PRINCIPLE

The preceding experiments show that the toxic principle contained in Derris kills insects both as a contact insecticide and as a stomach poison. It now remains to be shown how this poison kills insects. This phase of the work involves a careful study of the physiological effects of the toxic principle on insects and of how it reaches the internal tissues.

PHYSIOLOGICAL EFFECTS

In the foregoing experiments it was observed that the various spray mixtures and powders were effective only when they came in actual contact with the insects tested. The following experiments were performed to determine whether they would kill insects without coming in actual contact with them. In these experiments only the powder from *Derris* sp. was used.

Ten small fall webworms, confined in an observation wire-screen case, were placed ⅛ inch above the surface of a strong mixture of Derris powder and water so that the exhalation and vapors from the mixture could pass freely through the wire screen. No effects on the insects were observed which could be attributed to the presence of the insecticide.

Fall webworms, ants (*Monomorium pharaonis* L.), various species of aphids, roaches, and the larvæ of *Prodenia ornithogalli* Guenée were confined in large, air-tight glass tubes with Derris powder so that they could not touch it. As a rule, the exhalation from the powder had little effect upon the confined insects. None of the webworms or larvæ of Prodenia died, and only a small percentage of the ants and aphids and only the recently hatched roaches succumbed.

Most of the aphids dusted with Derris powder fell within a few hours in a paralyzed condition from the plants bearing them, and then they lay more or less helpless for a few hours before they died. Aphids sprayed with Derris mixtures and extracts behaved almost normally and showed no symptoms of paralysis; in short, they died very slowly and their behavior was similar to that of those sprayed with quassia extracts, described by McIndoo and Sievers (*7, p. 523*). Honeybees fed extracts of Derris seemed to die of motor paralysis; and their behavior was similar to that of those fed nicotine, described by McIndoo (*6, p. 91*); but it was somewhat different from the behavior of those fed arsenic.

HISTOLOGICAL METHODS OF TRACING DERRIS POWDER AND SPRAY MIXTURES IN INSECTS

Small individuals of fall webworms, caterpillars of Datana, silkworms, and cockroaches, confined in wire-screen observation cases, were dusted with Derris powder (No. 200). Three hours later all of them were "stupid," and after being removed from the cases they were put in vials containing thick celloidin. After remaining in the celloidin an hour they were put in other vials containing chloroform. Then an hour later they were cut into

small pieces and were fixed in a liquid containing equal parts of absolute alcohol and chloroform with corrosive sublimate to excess. The thick celloidin completely covered the integuments of the dusted insects and held the particles of powder where they were already adhering to the hairs and integuments. It did not pass into the mouth, anus, or spiracles but ran into all of the crevices and surrounded the hairs. The chloroform soon made the celloidin hard, thereby forming a hard layer around the insect, and thus holding the powder in position. Sections made from this material were stained with eosin in equal parts of absolute alcohol and chloroform. This method kept the celloidin hard and thus firmly held the particles of powder in position.

A study of the sections described above showed the following: A thick layer of celloidin, dotted with particles of powder, completely surrounded the integument, and processes from it ran into all of the crevices or indentations of the integument. The heat in the paraffin bath caused the celloidin to shrink, thereby drawing it away from the integument at places; but at other places it remained in contact with the integument. Most of the powder in the layer of celloidin lay against the integument and none could be seen inside the insect, except particles here and there which seemed to have been dragged inside by the microtome knife; none was seen in the tracheæ and only occasionally was a small amount observed in the spiracles, but never enough to clog them.

To be able to trace the powder better and distinguish it from the particles of food in the intestine, the following experiments were performed: Eight fall webworms were dusted with a mixture of Derris powder and lamp-black, and eight more with a mixture of Derris powder and carmine; the lamp-black and carmine were finely pulverized and were mixed thoroughly and in equal proportions with the Derris powder. The first four of each set were three hours later fixed intact in the modified Carnoy's fluid (equal parts of absolute alcohol, chloroform, glacial acetic acid, and corrosive sublimate to excess); and the second four of each set were treated by the celloidin process, described above. Many sections were made from the material of each set; one-half of those from the material dusted with the Derris powder and lamp-black mixture were stained in the mixture of absolute alcohol, chloroform, and eosin; and the other half were left in the paraffin-ribbon stage on the slides and not stained. The sections from the material dusted with the Derris powder and carmine mixture were likewise treated, one-half being stained with methylin blue in 95 per cent alcohol and the other half being left unstained in the paraffin-ribbon stage.

A study of these sections showed the following: The black and red powders were easily traced around the outside of the integuments but never in the tracheæ, and only occasionally did a small amount lie in the mouth of a spiracle. In many of the sections, small masses of the

colored particles lay inside the integument; but most of them, if not all, seemed to have been dragged there by the microtome knife, or washed there by the staining liquid and xylol. However, a careful study of the paraffin-ribbon sections, from the material dusted with the Derris powder and carmine mixture, showed red powder only on the outside of the integument and none inside, except a small amount here and there in the intestine.

McIndoo (*6, p. 103*) has shown that nicotine spray solutions not containing soap do not pass into the tracheæ of certain aphids and caterpillars, and the same is true for quassia-spray solutions not containing soap. Quassia-spray solutions containing soap, however, pass freely into the tracheæ and finally reach the various tissues (*7, p. 525*). In view of these results it was not considered necessary to trace Derris extracts contained in water and in soap solution.

The preceding histological study seems to show the following: Derris powder dusted upon insects does not pass into the tracheæ, but a limited amount of it may lodge in the spiracles, though never sufficiently to interfere with breathing. In order that the vapors and exhalation from a nicotine-spray solution be effective, it is necessary for the insects sprayed to carry some of this solution on their bodies; likewise it is necessary for the insects dusted with Derris powder to carry some of this powder on their bodies in order that its exhalation may pass into the spiracles in as undiluted a condition as possible. After being dusted the insects seem to swallow some of the powder, which later may act as a stomach poison. Soap solutions containing Derris extracts pass freely into the spiracles and finally reach the various tissues, but probably the extracts kill by first affecting the nerve tissue.

SUMMARY

Derris, known widely as a powerful East Indies fish poison, was found to fulfill several of the requirements of a general insecticide; it acts both as a contact insecticide and as a stomach poison, but is of no practical value as a fumigant. Six species of Derris were tested, but only two of them (*elliptica* and *uliginosa*) were found to be satisfactory for insecticidal purposes.

According to the views of various authors, the toxic principle in Derris is a resin, which affects the various classes of animals according to the development of their nervous systems. It kills some insects easily and others with difficulty, but it usually acts slowly and seems to kill by motor paralysis.

Denatured alcohol was found to be a good economic solvent for extracting the toxic principle, which when applied in spray mixtures proved to be efficient against certain aphids, potato-beetle larvæ, and small fall webworms. For proprietary insecticides it is possible to incorporate the extracts from Derris into soft soaps which when greatly diluted with water are ready for use.

Derris powder, used as a dust under practical conditions, was found to be efficient against dog fleas, chicken lice, house flies, three species of aphids (*Aphis rumicis* L., *Aphis pomi* De Geer, and *Myzus persicae* Sulz.), potato-beetle larvæ, and small fall webworms, but of no practical value against bedbugs, roaches, chicken mites, mealybugs, *Orthezia insignis*, red spiders, or against the crawling young of the oyster-shell scale. Used as powder in water with or without soap under practical conditions, it proved to be efficient against most of the aphids sprayed and also against cabbage worms (*Autographa brassicae* Riley), the larvæ of apple datanas (*Datana ministra* Dru.), oak worms (*Anisota senatoria* S. and A.), small tent caterpillars, and potato-beetle larvæ.

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EFFECTS OF HEAT ON TRICHINÆ

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INTRODUCTION

It is a well-known fact that the larvæ of *Trichinella spiralis*, which are of rather common occurrence in pork, may be killed by thorough cooking and the meat thereby rendered safe for food so far as concerns the danger of trichinosis. As to the actual temperature required to kill the parasites, however, various writers give very different figures, so that the question of the thermal death point has been rather uncertain.

The thermal death point of trichinæ is a matter of great practical importance in connection with the control of cooking processes employed by meat-packing establishments in the preparation of cooked products containing pork. The simple rule of cooking pork until it is well done, which can be applied satisfactorily by a careful cook in the household kitchen, is not suited to conditions in meat-packing establishments. Instead of such a rule a more exact statement of requirements is desirable. In fact, the Bureau of Animal Industry, which is charged with the enforcement of the federal meat-inspection law, requires that pork or products containing pork cooked in establishments operating under Federal inspection shall be heated sufficiently to insure a temperature throughout all portions of the meat that will destroy the vitality of any trichinæ which may be present, specifically a temperature of 137° F. (58° + C.). This temperature is several degrees higher than the temperature that has been accepted by the bureau as representing the thermal death point of encysted trichinæ, but the difference between the two represents no more than a reasonable allowance as a margin of safety.

Before a decision could be reached as to the degree of heat required to destroy the vitality of encysted trichinæ, it was found necessary to supplement the investigations on this question which are recorded in the literature with further experimental work; and it is the purpose of this paper to set forth the results obtained. This work was begun by the senior writer in 1913, continued in 1914 and 1915, and in the latter part of 1915 taken up by the junior writer.

REVIEW OF LITERATURE

Haubner, Küchenmeister, and Leisering (*5*) [1] state that trichinæ are killed by prolonged salting, followed by 24 hours of smoking, but do not give data as to the temperature of smoking.

[1] Reference is made by number (italic) to "Literature cited," pp. 220-221.

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Fiedler (*1, p. 26-29*) found that if small particles of trichinous meat were heated to 35° R. (43.75° C.) in water the heating had no other effect than to render the parasites more active when viewed at the same temperature under the microscope. Similar results were obtained by heating to a temperature of 40° R. (50° C.). The trichinæ in finely chopped meat held at a temperature of 50° R. (62.5° C.) for 15 minutes and then cooled were found to show movement when gently warmed, but reexamination of the meat 24 hours later failed to show any trichinæ that would move when warmed. This experiment was frequently repeated with similar results, and similar results were obtained with a temperature of 52° R. (65° C.). Temperatures of 58° R. (72.5° C.) and upward, allowed to act for a period of 10 minutes in all cases, affected the parasites so that no movement occurred afterward when gentle heat was applied. Three rabbits and a cat were fed trichinous meat after it had been heated 10 minutes at a temperature of 50° R. (62.5° C.), and none became infected. Trichinous meat heated 10 minutes at a temperature of 40° to 42° R. (50° to 52.5° C.) infected a rabbit. In another experiment meat heated at 40° R. (50° C.) for 10 minutes failed to infect a young cat. Trichinous meat heated at 60° R. (75° C.) for 10 minutes failed to infect two rabbits.

In another paper Fiedler (*2, p. 467-468*) reported an experiment in which he fed two rabbits with minced trichinous meat that had been heated in water for 10 minutes at a temperature of 50° R. (62.5° to 65° C.). No infection resulted. He also reported an experiment in which two rabbits were fed with trichinous meat that had been heated in water for 10 minutes at a temperature of 45° to 46° R. (56.25° to 57.5° C.). No infection resulted.

Haubner (*4*) states that the smoking of pork at a temperature which reaches and exceeds 52° R. (65° C.) kills the trichinæ or brings about their early death.

Rodet (*12*) states that trichinæ do not die at a temperature of 55° to 60° C. He also asserts that they survive even a temperature of 70° to 80° C. and succumb with certainty only to a temperature of 100° C. In support of his views Rodet presents very imperfect experimental evidence. He states that he placed pieces of trichinous muscle in water at a temperature of 70° to 80° C. and allowed them to remain there for some time. Upon being taken out of the water the trichinæ in the meat were still lively. When plunged into water at 100° C. they were killed and became completely uncoiled.

Fjord and Krabbe (*3*) concluded that encysted trichinæ die at 52.5° C. after a 30 minutes' exposure. At 54° C. they survived 10 minutes and at 55° to 56° C. they died in 5 minutes. Their method of procedure consisted in cutting up trichinous meat and heating it in a vessel containing warm water while agitating the contents with a thermometer. To

determine the effects of the heating upon the vitality of the parasites they fed the meat to rabbits, which were examined for trichinæ 15 to 30 days after feeding.

Perroncito (7) records observations on the behavior of the larvæ under the influence of high temperatures and draws the conclusion that a temperature of 48° to 50° C. is sufficient to kill the parasites. He placed decapsuled larvæ as well as encysted larvæ in salt solution and examined them on a warm stage. He observed that as the temperature increased the larvæ became more active, but that at 45° C. their activities ceased. If the temperature was lowered they resumed their activities. If the temperature was raised to 48° or 50° C. they became completely inactive and remained so even when the temperature was lowered.

Vallin (*13*) records a series of experiments on the effects of heat on trichinæ. He heated small pieces of trichinous meat in tubes containing water, placed the tubes on a sand bath, and read the temperatures on a thermometer with which each tube was provided. He found that a 20-minutes' exposure to a temperature of 60° C. resulted in a complete destruction of the vitality of the larvæ. He fed the heated meat to two rabbits and four guinea pigs and failed to infect them. Vallin states that temperatures below 60° C. are uncertain in their effects, since after heating meat to 56° C. he succeeded in infecting with it one guinea pig, although two rabbits to which the meat was fed escaped infection. He tried temperatures lower than 56° C. and found them ineffective.

Leuckart (*6*) states that *Trichinella spiralis* does not perish until it is acted on by a temperature ranging between 62° and 69° C.

Piana (*8*) concluded as a result of certain experiments that a temperature of 56° C. is fatal to the larvæ of *Trichinella spiralis*.

Ransom (*10, p. 159*) states:

> With reference to the effects of high temperatures upon the vitality of trichinæ, various statements are found in the literature which seem to have for the most part rather imperfect experimental evidence as a basis. From a rather small series of experiments conducted within the last two years, I have found that encysted trichinæ regularly die when exposed for a short time to a temperature somewhere between 53° and 55° C.

The earlier of these experiments supplied the data upon which was based the following statement (*9*): "The results already obtained in the investigations . . . show that the parasites die after a brief exposure to a temperature between 53° and 55° C."

Winn (*14*) records a series of experiments in which trichinous meat was heated to certain temperatures, maintained at those temperatures for 15 minutes, and then fed to experimental animals. The effect of the heat was judged by the degree of infection as compared with that of animals fed on similar quantities of meat which were unheated. Winn found that temperatures below 53° C. produce no apparent effect upon

the vitality of the worms. At 53° C. he found the vitality of the worms slightly reduced, but the results were variable. At 54° C. there was a further reduction in vitality, but meat which was heated to 55° C. and maintained at that temperature for 15 minutes was not capable of producing an infection.

EXPERIMENTAL WORK

Experiments by the present writers on the effects of heat on the larvæ of *Trichinella spiralis* have been made with meat containing encysted larvæ as well as with larvæ freed from their capsules by artificial digestion. In the former case there is more or less difficulty in obtaining accurate data, since the temperature in the interior of the meat does not necessarily correspond to the temperature of the medium in which it is heated. This difficulty may be overcome, however, if small pieces of muscle tissue are used and if the temperature is raised gradually. In experiments on larvæ freed from their cysts by artificial digestion more accurate determinations can be made, since the temperature of the medium is an excellent index to the temperature of the parasites themselves. From a comparison of the results obtained by the two methods definite conclusions regarding the thermal death point of the larvæ may be drawn.

OBSERVATIONS ON THE SURVIVAL OF DECAPSULED LARVÆ IN VARIOUS MEDIA

In connection with experiments on the effects of heat upon decapsuled larvæ, the question of their survival in various media following artificial digestion is important, since such experiments are complicated by the factor of abnormal environment, and results obtained might not correspond with those obtained in experiments in which the parasites are subjected to heat while still inclosed in their capsules in pieces of meat. Encysted trichinæ may be kept alive for many months and may still be viable in meat that has become badly decomposed. Although decapsuled larvæ are unlikely to survive as long as encysted larvæ, they can be kept alive for considerable periods of time. In a paper by the senior writer (Ransom, *11*), it has been shown that decapsuled larvæ may retain their normal activity and appearance when kept in tap water or 0.6 per cent salt solution at a temperature of about 20° C. for a period of from 10 days to two weeks or more, and that they have been kept alive and very active for as long as 11 days in 2 per cent salt solution. On the other hand, at a temperature of 38° decapsuled larvæ kept in tap water became inactive within a few hours, whereas when kept in 0.6 per cent salt solution at the same temperature for the same length of time they suffered no apparent injury.

Further observations have been made by the junior writer which show quite clearly that the longevity of the larvæ after artificial digestion depends upon both the medium in which they are kept and the temperature

to which they are subjected. Pure water as compared to physiological salt solutions was found to be distinctly injurious, the injurious action varying directly with the temperature. Larvæ kept in distilled water at a temperature of 39°–40° C. were all dead at the end of 22 hours, while in 0.7 per cent solution of sodium chlorid or in Ringer's solution they lived longer, although they all died within 48 hours. In distilled water at a temperature of 32°–33° decapsuled larvæ were nearly all uncoiled at the end of 48 hours, while in 0.7 per cent sodium-chlorid solution or in Ringer's solution some were still alive at the end of 5 days. Similar differences were observed in the case of lower temperatures. In distilled water at 25°–26° larvæ remained alive for 4 days; in physiological salt solutions at 25°–27° some were still alive at the end of 13 days; in distilled water kept at a temperature of about 8° only a few larvæ were still alive at the end of 12 days; while in physiological salt solutions at the same temperature some larvæ were still alive at the end of 50 days.

From these observations and our general knowledge of the phenomena of osmosis it would appear that the loss of salts from the tissues of the worms into the water and the penetration of the water into the tissues of the worms are important factors in bringing about the death of the worms when kept in hypotonic media, such as distilled water. This belief is borne out also by the fact, noted in a former paper (*11, p. 849*) and repeatedly observed since that paper was written, that larvæ kept in a hypotonic solution until they have begun to show distinct evidence of its effects, such as loosening of their coils and paling of their protoplasm, if transferred to a physiological salt solution before the injurious action of the hypotonic medium has gone too far, will usually resume a normal state of contraction and a normal or almost normal brown color. Another indication that the death of decapsuled larvæ kept in hypotonic solutions may be dependent upon osmotic processes is that they die more quickly at high than at low temperatures, which is in harmony with the fact that osmosis is hastened by raising the temperature.

Another factor or factors, however, enter into the matter, inasmuch as in isotonic solutions as well as in hypotonic solutions the larvæ do not survive so long at high temperatures as at low temperatures. It may be supposed that at the higher temperatures death of the larvæ kept in isotonic and comparatively inert solutions is brought about by exhaustion resulting from the greater activity of the worms and consequently more rapid oxidation of their tissues than at lower temperatures. Such an explanation is complicated by the fact that larval trichinæ encysted in the muscles of a living animal may live for many years, although constantly subjected to a temperature at which they live only two or three days when removed from their cysts and kept in salt solutions. Possibly in the living animal they are kept in a relatively inactive condition through the operation of factors no longer present when they are removed

from their normal environment, and it is possible also that they may be able to replace waste through the absorption of nutritive materials from their host.

A natural corollary to experiments on the effects of hypotonic solutions are experiments on the effects of hypertonic solutions. A typical example of such an experiment is one in which decapsuled larvæ were kept for 22 hours in a molar solution of dextrose. At the end of this time they were found to be partially uncoiled; their protoplasm was dull in appearance; the cuticle was wrinkled, particularly in the posterior portion of the body; the body wall was wrinkled; and the cells of the esophagus were indistinct. After having been transferred to and kept in 0.7 per cent salt solution overnight, they were found to be tightly coiled and normal in appearance. Similar results were obtained in a repetition of this experiment.

So far as concerns the purposes of the present paper, the foregoing observations are of interest because they show that trichinæ freed from their cysts by artificial digestion may be kept alive for a long time in physiological salt solutions, in water, and in certain hypertonic solutions, and that, although within a temperature range the upper limit of which does not exceed 40° C. their longevity decreases as the temperature at which they are kept is raised, they do not in any case die quickly.

EXPERIMENTS WITH DECAPSULED LARVÆ

Inasmuch as trichina larvæ that have been freed from their cysts by digestion of finely chopped trichinous meat in artificial gastric juice[1] at a temperature of 38° to 40° C. for a period of about 20 hours can be kept alive for long periods of time, they can be conveniently used in experiments on the effects of heat. In a medium such as a 0.6 per cent or 0.7 per cent solution of sodium chlorid, but also in plain water if not kept too long, they display more or less activity even at ordinary room temperatures but commonly assume a posture in which they are tightly coiled spirally; and their movements are often limited to a tightening or loosening of the coil. Their protoplasm, when unaffected by heat or other injurious agents, exhibits a certain brilliancy in appearance; and pigment in the cells of the alimentary tract, especially of the esophagus, gives them a distinct brownish color. After a little experience, departures from the normal both as to their behavior and appearance of their protoplasm can easily be detected by microscopic examination. As a rule, in experiments in heating decapsuled larvæ, the larvæ were placed in a beaker or test tube containing sometimes water but usually a phys-

[1] The following fluid has yielded satisfactory results:

Scale pepsin (U. S. P.)	2.5 gm.
Sodium chlorid	2 gm.
Hydrochloric acid (sp. g. 1.19)	10 cc.
Water	1,000 cc.

iological salt solution or Ringer's fluid; and this was heated to the desired temperature on a water bath over an open flame, or in an incubator. After being cooled, individuals were removed with a pipette to hollow ground slides, or in some cases transferred to a Petri dish or shallow stender dish and allowed to cool. They were then examined directly on a warm stage, either on slides or in the dishes, in order to determine the results of the experiment.

BEHAVIOR OF DECAPSULED LARVÆ WHEN HEATED

When trichina larvæ are heated on a warm stage their reactions may be directly observed with the microscope. As the temperature rises they begin to uncoil and become very active, their activity gradually increasing. When the temperature has reached the neighborhood of 50° C. spasmodic contractions are commonly observed, and the larvæ twist themselves into various shapes. With a further rise of temperature they grow sluggish and may become either uncoiled and inactive or else tightly coiled and quiescent. After passing into this sluggish condition they may again become lively if the temperature is lowered, but if subjected to a sufficiently high temperature for a sufficient length of time they do not recover when removed to a cool place.

Decapsuled trichinæ killed by heat usually become uncoiled and assume a characteristic shape resembling the figure 6. If allowed to stand for some time the protoplasm becomes dull, certain granulations appear, and often the cell partitions in the gonads can no longer be distinguished. Larvæ in this condition are readily recognizable as dead. Sometimes, however, larvæ that have been subjected to heat may remain loosely coiled and the protoplasm may not undergo any conspicuous changes. From experience it has been learned that larvæ in this condition are usually dead. A generally satisfactory test of life is heat stimulation; if still viable the larvæ will usually uncoil and move. Even individuals with a minimum amount of vitality will move the anterior or posterior end very sluggishly. However, the most reliable test of life, or at least of their viability from a practical standpoint, is feeding them to experimental animals and thus determining their ability to reproduce; and this has been done in some instances but not so regularly as in experiments on encysted trichinæ.

DETAILS OF EXPERIMENTS

Some experiments on the effects of heat on decapsuled trichinæ were made by the senior writer in 1913, 1914, and 1915, after which the work was taken up by the junior writer and continued along the same general lines.

EXPERIMENT 1 (April 5 and 7, 1913).—A decapsuled larva was sealed under a cover glass in salt solution on a slide and heated to 54° C. on a

warm stage. The temperature was held at 54° for a few moments. The worm was inactive at this temperature but resumed its movements when the slide was cooled. The same worm was reheated to 55° and became entirely motionless at this temperature. The temperature was raised to 55.5° and the slide then cooled. The worm became active again on cooling.

Another decapsuled larva was heated in the same manner. It became sluggish in its movements and coiled up at a temperature of 48° C. The temperature was raised slowly to 56°, and the slide was allowed to cool as soon as this temperature was attained. The worm resumed its active movements when cooled. In order to check the correctness of the temperature indicated by the thermometer in this experiment, some crystals of diphenylamin having a melting point of 54° were placed on a slide under a cover glass and heated on the stage. They melted when the thermometer registered 54°. A second trial gave the same result.

On April 7, a decapsuled larva was heated as described above. The temperature was raised slowly to 56° C. and then held for five minutes at 56° to 56.5°. When cooled the worm did not resume its movements, its internal structure showed slight disorganization, and it was undoubtedly dead.

EXPERIMENT 2 (March 28, 1914).—Decapsuled trichinæ, isolated by artificial digestion from a mixture of meat from three trichinous rats, were heated in a beaker of constantly stirred water over a hot water bath to a maximum of 53.6° C., 10 minutes being required for the temperature to rise to this point from 30°. The temperature dropped to 46.2° in another 10 minutes, after which 233 larvæ were examined at room temperature. All were inactive. Unheated larvæ from this lot when examined at room temperature were active. Another lot of larvæ from the same source was heated in the same manner, the temperature rising from 20° to 51° in 21 minutes, and then dropping in 6 minutes to 45.8°. One hundred and thirty-nine larvæ were then examined at room temperature, and 65 of them were found to be inactive. Of the 74 active larvæ, all but 2 were sluggish. A third lot of larvæ from the same source was heated in the same manner from 24° to 50° in 12 minutes, and then cooled to 46° in 6 minutes. Out of 159 examined, 18 were inactive. Some of the 141 active larvæ were sluggish.

EXPERIMENT 3 (May 16, 1914).—Decapsuled larvæ, isolated by artificial digestion from a mixture of meat from two trichinous rats, were heated in a beaker of constantly stirred water over a water bath. The temperature was raised from 23° to 48.4° C. in 8 minutes and held at 48.4° 1 minute. The beaker was then allowed to cool. One hundred and ten larvæ were examined on a warm stage. Thirty-five were inactive and 75 active, mostly very lively. Another lot of larvæ from the same source was heated in the same manner from 22° to 51° in 10 minutes.

Examination of 213 larvæ on a warm stage showed 179 inactive and 34 active, most of them very lively. Another lot was heated from 30° to 51.9° in 10 minutes. Ninety-nine were examined, and of these 93 were inactive and 6 active. Another lot was heated from 30° to 53° in 4 minutes. One hundred and eighteen were examined, and of these 72 were inactive and 46 active, sluggish. Another lot was heated from 22° to 53° in 12 minutes. One hundred and forty-seven were examined, and of these 109 were inactive and 38 active, sluggish. As a control upon the results of this experiment 158 unheated larvæ from the same source as those subjected to heat were examined on a warm stage. Of these 22 were inactive and 136 active.

EXPERIMENT 4 (November 17, 1914).—Decapsuled larvæ, isolated by artificial digestion from the meat of a trichinous hog, were heated in a beaker of water over a hot water bath for a period of 10 minutes, during which time the temperature gradually increased from 23° to 53.4° C. The beaker was then cooled. Seventeen of the larvæ were examined on a warm stage and one was observed to move slightly. Fifteen minutes later the larvæ remaining in the beaker were reheated to a temperature of 53.6° C., seven minutes being required to raise the temperature to this point from 38°. Twenty-four larvæ were examined after this reheating; one exhibited definite movements on a warm stage. The others were more or less tightly coiled and presumably still alive. Thirteen minutes later the larvæ remaining in the beaker were heated a third time, the temperature being raised rapidly (in 3 minutes) from 43° to 55°. Thirty-nine larvæ were examined; all were motionless and failed to react to heat, evidently dead.

EXPERIMENT 5 (November 17, 1914).—Decapsuled larvæ from the same source as those used in Experiment 4 were heated in the same manner from 16° to 54° C., 7½ minutes being required for raising the temperature. Twenty-three larvæ were examined after heating and all were found to remain inactive on a warm stage. The remainder of the larvæ in the beaker were left on the laboratory table until the following day when 42 of them were examined on a warm stage heated to 45°. Most of these were inactive but more or less tightly coiled. Thirty-five others were placed on a warm stage heated to 61°. Six of these exhibited convulsive movements before they succumbed to the heat, the others showing no response to stimulation.

EXPERIMENT 6 (November 17, 1914).—Decapsuled trichinæ, isolated by artificial digestion from a mixture of meat from six trichinous hogs, were heated in a beaker of water over a hot water bath to a temperature of 53.4° C. Some of them showed signs of life when examined on a warm stage. The beaker was reheated to 55°. Fifty larvæ were then examined on a warm stage and all were found to be dead.

EXPERIMENT 7 (December 19, 1914).—Decapsuled larvæ, isolated by artificial digestion from meat of a trichinous hog, were heated in 0.6 per cent salt solution in a corked bottle over a water bath. The temperature, determined by a thermometer inserted through the cork, rose from 24.4° to 56.7° C. in 44 minutes and remained at this maximum for 30 seconds, after which the bottle was allowed to cool, the temperature dropping to 34.4° in 38 minutes. Three hundred and sixty-five of the larvæ were then examined on a warm stage and all were found to be inactive. As a control on the results of this experiment 22 unheated larvæ from the same source were examined on a warm stage; 4 were inactive, 18 active.

EXPERIMENT 8 (April 6, 1915).—Decapsuled trichinæ, isolated by artificial digestion from a mixture of meat from six hogs, were kept 7 days in 0.6 per cent salt solution at ordinary room temperature. Some were then heated in a beaker of the salt solution, constantly stirred, over a water bath. The temperature rose from 20° to 54° C. in 7 minutes, and remained at this maximum for 30 seconds, after which the beaker was allowed to cool. Examination of some of the larvæ from the beaker showed that most of them were more or less uncoiled, but some were tightly coiled and practically normal in appearance. The beaker was kept until the following day at ordinary room temperature and the contents again examined. The great majority of the worms were still alive, but most of them were not tightly coiled.

Another lot of larvæ from the same source was heated in a similar manner but more slowly, the temperature rising from 23° to 54.8° C. in 56 minutes, remaining at 54.8° for 1 minute, after which the beaker was allowed to cool. Four hundred and seventy larvæ were examined; all were uncoiled, and their protoplasm was rather dull in appearance. The beaker was kept at room temperature until the following day, when examination of 200 larvæ showed that all were dead.

Subsequent experiments on the effects of heat on decapsuled larvæ were performed by the junior writer.

EXPERIMENT 9.—Decapsuled trichinæ in a physiological salt solution were placed in a test tube and a thermometer immersed in the solution. The test tube was placed in a beaker of water, which was heated rapidly until the thermometer registered 55° C. This temperature was attained in four minutes. The contents of the test tube were immediately transferred to a stender dish and allowed to cool. The larvæ were then examined. Nearly all were unaffected. A few days later this experiment was repeated, increasing the time of heating to about eight minutes. Similar results were obtained.

The results of other experiments with various lots of decapsuled larvæ are shown in the following table:

TABLE I.—*Effect of various temperatures on decapsuled larvæ*

Maximum temperature.	Time required to reach maximum temperature.	Results.
°C.		
53	Not recorded	Some alive.
53	50 minutes	Nearly all alive.
54	Not recorded	Some alive.
54	do	Do.
54	42 minutes	All dead.
54.6	54 minutes	Do.
54.8	Not recorded	A few showing sluggish movements.
55	do	None active.
55	do	Do.
55	77 minutes	All dead.
55	60 minutes	All expanded.
55	65 minutes	Do.
55	37 minutes	All dead.
56	Not recorded	Do.
56	52 minutes	Do.
56	83 minutes	Do.

EXPERIMENT 10.—A 0.6 per cent salt solution was heated to 56° C. At this point some decapsuled larvæ were spurted into the solution from a capillary pipette. The temperature dropped from 56° to 55° in 75 seconds, and the contents of the vessel were then emptied into a shallow dish and examined. Of 25 larvæ, 14 were uncoiled and 11 tightly coiled. The same experiment was repeated. Of 21 larvæ, only 3 were completely uncoiled. In another test the larvæ were spurted into the solution at 55° after which the temperature was allowed to drop to 54°, which required 85 seconds. On examination following transfer to a shallow dish, only 3 out of 18 larvæ were found to be completely uncoiled.

In order to control the results of direct examination of decapsuled larvæ after heating, the junior writer in two instances fed some of the larvæ to rats. Thus larvæ heated rapidly to 55° C. in Experiment 9 were fed to two rats, which when killed at the end of a month were found to be moderately infected, a result in agreement with the results of direct examination of the larvæ. In another case—one of the experiments summarized in Table I—larvæ heated gradually for 60 minutes to 55° were fed to two rats, which were found free from trichinæ a month later. Another rat fed unheated decapsuled larvæ from the same source became infected.

From the foregoing experiments it is evident that decapsuled trichina larvæ are killed by a temperature of 55° C., provided this temperature is gradually attained. Many may be killed by lower temperatures, but the results of heating to temperatures lower than 55° are uncertain. It is also apparent that a momentary exposure to a temperature of 55° is not sufficient to destroy the vitality of decapsuled larvæ, as is shown by the results of Experiments 1, 9, and 10.

EXPERIMENTS WITH ENCYSTED LARVÆ

The experiments on decapsuled larvæ were supplemented by experiments on encysted larvæ in their natural location in pieces of infested muscle, the earlier of these experiments being made by the senior writer, the later, as noted, by the junior writer.

EXPERIMENT 11 (March 31, 1913).—Small pieces of meat from a trichinous rat were placed in a beaker of water (about 500 cc.) in a constant-temperature oven. The temperature of the water increased from an initial temperature of 18.4° to 48.4° C. in 1 hour and 10 minutes, at which time a piece of the meat was removed. Ten minutes later, when another piece was removed, the temperature had reached 51°. Eleven minutes after this at a temperature of 52.8° another piece was removed. After another period of 15 minutes, when the temperature had reached 55°, another piece was removed. Thirty-seven minutes later, when the thermometer registered 59.8°, another piece of meat was removed. A few larvæ were isolated by dissection from these various pieces of meat and examined under the microscope. The larvæ from the pieces heated to 48.4° and 51° were alive and active. One out of four larvæ from the piece heated to 52.8° showed slight movements; the others were inactive. Those from the pieces heated to 55° and 59.8° were inactive when examined. The results of direct examination of the larvæ were checked by feeding the various pieces of meat to guinea pigs. The guinea pigs fed with the meat which had been heated to 48.4° and 51° became heavily infected; those fed the pieces heated to 52.8°, 55°, and 59.8° remained free from trichinæ.

EXPERIMENT 12 (April 1, 1913).—Several small pieces of rat muscle were placed in a vessel containing 500 cc. of water and heated in an oven from an initial temperature of 16° to a temperature that reached 55° C. at the end of two hours. Pieces of meat were removed at temperatures of 51.2°, 52.2°, 53°, and 55°. A few larvæ from each piece of meat thus removed were isolated and examined directly on a warm stage. Samples from these pieces of meat were also fed to guinea pigs, which were killed about a month after feeding. The direct examination of the larvæ on a warm stage showed that, with the exception of those from the meat heated to 55°, the majority were alive and responded to thermal stimulation. Those heated to 55° were loosely coiled and did not become active on the warm stage.

The results of the feeding experiments were as follows: The guinea pig that was fed meat heated to 51.2° C. was killed seven days after feeding because it became sick. The muscles were negative, but one pregnant female trichina was found in the intestine. The guinea pig that was fed meat heated to 52.2° was killed about five weeks after feeding, and only one encysted larva was found in the diaphragm. No parasites were found in the intercostal muscles. The guinea pig that was fed meat

heated to 53° was killed five weeks after feeding and was free from parasites. The meat heated to 55° also failed to infect two guinea pigs.

EXPERIMENT 13 (April 1, 1913).—Small pieces of meat from a trichinous rat were heated as in the previous experiment; but an open flame was used instead of an oven and the temperature was allowed to go up very rapidly, the water in the beaker meanwhile being stirred constantly. Meat heated from 27.8° to 53° C. in 3½ minutes was fed to a guinea pig and resulted in a mild infection. Meat heated from 27.8° to 52° in 3 minutes and from 20° to 49.2° in 6 minutes when fed to guinea pigs produced heavy infections.

EXPERIMENT 14 (April 3 and 4, 1913).—A small piece of meat from a trichinous rat was heated in a beaker of water which was constantly stirred. The temperature rose from 17° to 53° C. in 13 minutes and remained between 53° and 53.6° for 2 minutes. One larva afterwards isolated by dissection was inactive except at the anterior end which moved slightly; another was active, though the appearance of its protoplasm was somewhat altered.

Another piece of meat was similarly heated from about 20° to 54° C. in about 10 minutes. Larvæ isolated by dissection were alive and active. Another piece was similarly heated from 28° to 53° in 11 minutes and remained in the water another minute, during which time the temperature rose to a maximum of 53.8°. Larvæ isolated by dissection were alive and active. Two pieces were heated from 28° to 55° in 13 minutes. One piece was held at a temperature of 55° for 1 minute, the other piece at the same temperature for 2 minutes. Trichinæ isolated by dissection from these pieces were inactive. Another piece of meat from the same rat was heated from 30° to 54° in 5 minutes and held at a temperature of 54° to 54.8° for 1 minute. Larvæ isolated by dissection were found to be inactive.

EXPERIMENT 15 (April 9, 1913).—Small pieces of meat from two trichinous rats were tied in a cloth around the bulb of a thermometer, which was immersed in a beaker of water and heated. The temperature was held at 54.6° to 54.8° C. for five minutes. Ten larvæ were afterwards isolated by dissection. All were inactive except one, which showed a very slight movement of its anterior end.

EXPERIMENT 16 (May 16 and 19, 1914).—Portions of the diaphragm of a trichinous rat were heated in a beaker of water stirred constantly over a water bath. Trichinæ were dissected out of the meat after heating and examined under the microscope at room temperature. A portion was heated from 24° to 54° C. in four minutes. Four larvæ examined; 1 inactive; 3 active, sluggish. Another portion was heated from 24° to 53° in 6 minutes. Ten larvæ examined; all active. Another portion was heated from 23° to 54° in 5 minutes. Twelve larvæ examined; 3 inactive; 9 active but very sluggish; appearance of protoplasm abnormal.

In the following tests portions of the diaphragm of another rat were heated. A portion was heated from 24° to 54° C. in 5½ minutes. Ten larvæ examined; 9 inactive; 1 active, very sluggish. A portion was heated from 24° to 52° in 4¾ minutes. Ten larvæ examined; all active, lively. A portion was heated from 24° to 58° in 3¼ minutes. Ten larvæ examined; all inactive. A portion was heated from 26° to 53° in 3½ minutes. Five larvæ examined; all active but not very lively. A portion was heated from 26° to 55° in 4 minutes. Twenty-three larvæ examined; 21 inactive; 2 active, very sluggish. A portion was heated from 24° to 52.6° in 9 minutes. Twelve larvæ examined; 2 inactive; 10 active, but very sluggish; appearance of protoplasm abnormal. A portion was heated from 23° to 52.9° in 2½ minutes. Eight larvæ examined; all lively. A portion was heated from 22° to 52° in 3¾ minutes. Twenty-four larvæ examined; all lively.

EXPERIMENT 17 (May 20, 1914).—Portions of the diaphragm of a third rat were heated as in Experiment 16, but more gradually. Examination was made as in Experiment 16. A portion was heated from 26° to 53° C. in 12½ minutes and cooled to 48.8° in 5 minutes. Sixteen larvæ examined; all active, but sluggish; appearance of protoplasm duller than normal. A portion was heated from 23.2° to 52° in 14 minutes and cooled to 46° in 7 minutes. Thirteen larvæ examined; all active, fairly lively but not as vigorous as unheated larvæ; no conspicuous change in appearance of protoplasm; larvæ not coiled as tightly as normal larvæ. A portion was heated from 23° to 55° in 16 minutes and cooled to 50° in 5 minutes. Fifteen larvæ examined; all inactive; protoplasm dull and dead in appearance. A portion was heated from 37° to 54° in 9 minutes and cooled to 49.4° in 6 minutes. Twenty-three larvæ examined; all active but very sluggish; protoplasm dull and dead in appearance. A portion was heated from 27° to 54° in 11¾ minutes and cooled to 49° in 5 minutes. Twenty-four larvæ examined; 16 inactive; 8 active but very sluggish; protoplasm dull and dead in appearance.

Experiments on encysted trichinæ were made by the junior writer as follows:

EXPERIMENT 18.—Small pieces of meat from a rat killed one month after infection with trichinæ were heated in a physiological salt solution to 52°, 53°, 54°, and 55° C., respectively, and then allowed to stand in a refrigerator for two days. The larvæ were then freed from their capsules by teasing out the meat, and examined directly. Those heated to 52° were still tightly coiled, although a number of loosely coiled larvæ were also seen. Most of the larvæ heated to 53° were uncoiled, but a few were coiled normally. Those heated to 54° and 55° were entirely uncoiled, dull in appearance, and failed to become active when warmed.

EXPERIMENT 19.—Larger pieces of meat from a trichinous hog were heated as in the experiment just described, kept in a refrigerator for two days, and then fed to mice. The post-mortem examinations yielded negative results in all cases.

The results obtained from the experiments in which pieces of trichinous meat were heated agree with the results of those in which the larvæ were first freed from their cysts by artificial digestion and then heated in water or physiological salt solution. The larvæ are killed if the meat is gradually heated to a temperature of 55° C., though some may escape if the temperature rises rapidly to 55° and soon falls again. They may survive a temperature of 54°; but meat which has been exposed to a temperature of about 53°, gradually attained, is likely to be non-infective.

It may be concluded that meat which has been heated so that the temperature throughout reaches 55° C. (131° F.) will be innocuous so far as concerns the possibility that persons eating such meat will become infected with trichinæ, inasmuch as under ordinary conditions of cooking the rise of temperature will be gradual enough to insure the destruction of the parasites if the temperature of the meat actually reaches 55° C. or higher. Under the regulations of the Bureau of Animal Industry the minimum temperature that must be attained throughout all portions of pieces of pork or products containing pork that are cooked in establishments operating under federal meat inspection has been fixed somewhat higher than 55° C., namely 137° F. (58.33° C.), which allows a margin of safety of several degrees above the temperature that has been shown by our investigations to be fatal to trichinæ.

THE EFFECTS UPON TRICHINÆ OF CONTINUED EXPOSURE TO HEAT AT TEMPERATURES BELOW THE THERMAL DEATH POINT

It has been shown that trichina larvæ are killed by brief exposure to a temperature of 55° C., gradually attained; and since they will not afterwards resume their activity when thus heated, this temperature may be considered the thermal death point. The vitality of the larvæ may be destroyed also by exposure to lower temperatures, provided the application of heat is long enough continued. In the former case it may be assumed that death results from irreversible coagulations of the protoplasm, in the latter case either as the result of coagulation changes which become irreversible if the heat acts for a sufficient period, or as the result of exhaustion following excessive activity to which the larvæ are stimulated by heat. We may, therefore, distinguish three ranges of lethal temperatures: The highest, in which death comes quickly from rapid and irreversible coagulations of the protoplasm; an intermediate range, in which death results probably from somewhat similar coagulation changes, changes, however, from which the parasites may more or less completely recover if the temperature is lowered before death occurs; and the lowest range, in which death is apparently brought about by exhaustion from increased activity.

The following experiments to determine the effects of the continued exposure of decapsuled larvæ to temperatures below 53° C. were carried out by the junior writer. The larvæ in 0.7 per cent salt solution or in Ringer's solution were first heated to a given temperature and then placed in an incubator at the same temperature for a given period. When taken out of the incubator the larvæ were kept at room temperature at least an hour before they were examined.

EXPERIMENT 20.—In one test the larvæ were all dead after exposure for three hours to a temperature of 48° C., but generally an exposure to a temperature of 48° for less then four hours failed to destroy their vitality. In every case, however, after they were heated four hours at a temperature of 48° they were all uncoiled, having assumed the shape of the figure 6; and they failed to react to heat stimulation.

EXPERIMENT 21.—When exposed to a temperature of 49° C. nearly one-half the larvæ in one lot were still alive at the end of two hours. Another lot from a different host animal succumbed to a similar treatment, but in no case did a briefer exposure to 49° prove effective. When subjected to 49° for 3¼ hours all the larvæ became completely uncoiled, rigid, and insensitive to thermal stimuli.

EXPERIMENT 22.—At a temperature of 50° to 50.6° C., the vitality of the larvæ was completely destroyed after an exposure of 1 hour and 20 minutes. At a constant temperature of 50° an exposure of 1½ hours proved fatal.

EXPERIMENT 23.—An exposure of one hour to a temperature of 52° C. was sufficient to destroy the vitality of decapsuled larvæ.

From the foregoing experiments it is evident that decapsuled trichina larvæ die in a comparatively short time when exposed to temperatures in the neighborhood of 50° C. and that the time required for their destruction increases as the temperature is lowered. If the results of these experiments are considered in connection with the question of the length of time that decapsuled larvæ survive at temperatures ranging below 40°, already discussed in this article, it may be concluded that between limits at which the larvæ become altogether quiescent because of the effects of heat on the one hand and of cold on the other their longevity varies inversely with the temperature. It would, however, not be safe to conclude from the experiments just described that exposure of trichina larvæ to the temperatures given for the stated periods of time would be sufficient in all cases to destroy the vitality of the parasites. It is not improbable that in these experiments the larvæ had already become somewhat exhausted as a result of abnormal activity during the process of artificial digestion, and furthermore it is possible that different lots of trichinæ vary considerably with respect to their store of vitality. The following experiments by the senior writer show that the vitality of encysted trichinæ as well as that of decapsuled

trichinæ may be destroyed by continued heating at temperatures lower than that which kills on brief exposure. Like the experiments with the decapsuled larvæ, however, they are not sufficiently extensive to allow definite conclusions to be drawn as to the periods of time necessary to insure the destruction of trichinæ exposed to temperatures lower than the thermal death point.

EXPERIMENT 24 (April 7, 1913).—A small piece of the diaphragm of the same rat which supplied the meat used in Experiment 14 was tied in a cloth around the bulb of a thermometer, which was immersed in a beaker of water heated to about 50° C. and the entire apparatus placed in a constant-temperature oven. The temperature, as indicated by the thermometer, varied from 50.2° to 51.6° during the two hours of heating the meat. Larvæ isolated from the meat by dissection were dead.

EXPERIMENT 25 (April 9, 1913).—Two small pieces of meat from the same rat used in Experiments 14 and 24 were tied in cloths around the bulbs of two thermometers and heated in a beaker of water as in Experiment 24. During the experiment the temperature, as indicated by the thermometers, varied between 49.6° and 50° C. One piece was removed after an hour's exposure. Two larvæ isolated from the meat by dissection were alive, but rather sluggish. The other piece was removed after an exposure of 1½ hours. Two larvæ were examined, one of which was dead, the other alive, but rather sluggish. Two guinea pigs were fed with the meat, but neither became infected. Another piece of meat from the same rat was similarly heated for one hour at a temperature of 50.1° to 50.4° C. A larva isolated from the meat after heating was alive and active. Another piece was similarly heated for 1½ hours at 50°. Five larvæ were isolated from the meat and examined. Four were certainly dead, the other inactive, but with protoplasm less changed than that of the others.

EXPERIMENT 26 (August 31, 1914).—Finely chopped meat from a trichinous rat was placed in water in a flask, which was kept 21 hours in an oven maintained at a temperature of 49° to 52° C. The temperature of the water during this time varied from 48.8° to 51.4°. Four larvæ dissected out of the meat after heating were dead. The meat was fed to two rats, both of which remained free from trichinæ. Some finely chopped meat from the same rat was heated 21 hours in a covered Petri dish in the same oven at a temperature of 49° to 52°. Five larvæ dissected out of the meat after heating were dead. Two rats to which the meat was fed remained free from infection.

EXPERIMENT 27 (September 3, 1914).—Finely chopped meat from a trichinous hog was heated in a closed jar in a constant-temperature oven for 19 hours. The temperature of the meat during this time varied between 47.8° and 48.4° C. Twenty-five trichinæ were dissected out of the meat after heating and all found to be dead.

EXPERIMENT 28 (September 8, 1914).—The eviscerated carcass of a trichinous rat was heated 17 hours in an oven at a temperature of 48° to 50° C. On removal from the oven the carcass had a bad odor; the upper surface was dried, the lower still moist. Twenty trichinæ were dissected out of the meat after heating and all found to be dead. Meat from the carcass was then fed to two rats, one of which remained free from trichinæ, while the other was found moderately infected when killed three months after feeding.

EXPERIMENT 29 (September 19, 1914).—Finely chopped meat from a trichinous rat was heated 5 hours in an oven at a temperature of 48° to 49° C. A few trichinæ afterward dissected out of the meat were shrunken, but their protoplasm was bright in appearance. After being soaked in water for 30 minutes some of the larvæ became lively, and 2 days later the remainder of the isolated larvæ kept in water at room temperature had also become active and normal in appearance. Some of the same meat was left in the oven until September 21, and thus exposed for 48 hours to a temperature of 48° to 49° C. It was hard and dry. Trichinæ isolated from the meat by dissection after it had been softened by soaking were very clear, pale, motionless, and apparently dead.

Additional data regarding the effects of the continued action of temperatures below the thermal death point were obtained by the junior writer. In these experiments, which are summarized in tabular form (Table II), the method of procedure was as follows: Meat from trichinous hogs was finely chopped by passing it through a meat chopper several times. A bottle with a capacity of about 200 cc. was half filled with the meat. Through a perforation in the cork a thermometer was inserted into the bottle and the top of the cork then paraffined. The bottle of meat was placed in a constant-temperature oven and the temperatures read on the thermometer in the bottle.

Inasmuch as the meat before being placed in the oven was kept in a refrigerator at a temperature of 8° to 10° C., a considerable period was required to bring its temperature near that of the oven. In nearly all the experiments shown in Table II the meat was in the oven about 2 hours before the first reading of the thermometer, given in the table as the minimum temperature, was made. Between the first and the final reading there was a slight fluctuation of the temperature but nearly always between the limits recorded in the table.

At the end of each experiment a portion of the meat was artificially digested in the usual way and the condition of the larvæ noted. As a control on the microscopic findings in each experiment two rats were fed portions of the meat, being given an average of about 10 gm. each. Unless they died earlier the test animals were killed about a month after feeding. The following table gives the record of 10 experiments:

TABLE II.—*Effects of continued action of temperatures below thermal death point on encysted trichinæ*

Temperature.		Period of exposure to given temperatures.	Appearance of larvæ after artificial digestion.	Results of feeding experiments.
Minimum.	Maximum.			
°C.	°C.	Hours.		
52	54	4	Coiled	Negative.
53	54.5	4½	Apparently dead	Do.
51	56	3⅙	Profoundly disorganized	Do.
52	54	4	Showing evidence of having been partially digested	Do.
50	52	4½	Uncoiled; evidently dead	Do.
49	50	5	Apparently dead	Do.
50	50	6	Probably dead	Do.
50	50	3½	Uncoiled and pale	Do.
52	53	6	.....do	Do.
50	54.8	4	.....do	Do.

From a practical standpoint the results of the experiments on the effects of continuous heating at temperatures below the thermal death point of trichinæ are of comparatively little importance so far as concerns the destruction of the vitality of trichinæ in fresh pork by cooking. Obviously, as compared to cooking at a higher temperature for a short time, there would be no advantage in subjecting meat to a lower temperature, which would require a very great lengthening of the period of heating. If for no other reason, the probable spoiling of the meat would preclude the use of such a method of destroying the vitality of the parasites. In connection with the preparation of certain kinds of cured pork products, however, the fact that heating at low temperatures for considerable periods of time is destructive to the vitality of trichinæ has been put to practical use. In this case there is also another factor which comes into play—namely, the destructive action of salt in hypertonic percentages, which increases greatly as the temperature increases. The question of the destruction of trichinæ in cured pork by heating at low temperatures will be discussed in another paper.

CONCLUSIONS

The vitality of the larvæ of *Trichinella spiralis* is quickly destroyed by exposure of the parasites to a temperature of 55° C., gradually attained, the result apparently of irreversible coagulation changes in the protoplasm. This temperature may be considered the thermal death point.

Trichina larvæ exposed to temperatures slightly below 55° C. for short periods of time may recover from this exposure; but they die if exposed for longer periods, recovery or death depending apparently upon whether or not beginning coagulation of the protoplasm has proceeded beyond a stage from which a return to normal may occur.

Exposed to temperatures in the neighborhood of 50° C., trichina larvæ die if the application of heat is sufficiently long continued, apparently as a result of exhaustion following excessive activity to which they are stimulated by the heat.

The longevity of trichina larvæ freed from their cysts by artificial digestion and kept at temperatures ranging between limits at which they become quiescent from the effects of heat and cold, respectively, varies inversely with the temperature.

Methods of destroying trichinæ by heating at temperatures below the thermal death point, which may be utilized in connection with the preparation of certain kinds of cured pork products, appear not to be applicable in the case of fresh pork.

Upon the basis of the results of experiments recorded in this paper the Bureau of Animal Industry has selected a temperature of 137° F. (58.33° C.) as the minimum temperature to which pork and products containing pork are required to be heated when cooked in establishments operating under federal meat inspection.[1] This temperature is several degrees above the thermal death point of trichina larvæ, thus providing a certain margin of safety.

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[1] This requirement has reference to the temperature actually reached in the interior of the meat and not merely to that of the water or oven in which it is cooked. It should also be understood that when meat is cooked for purposes of sterilization because of conditions other than trichinosis a higher temperature is necessary than that sufficient to destroy trichinæ.

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EFFECT OF REMOVING THE PULP FROM CAMPHOR SEED ON GERMINATION AND THE SUBSEQUENT GROWTH OF THE SEEDLINGS

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INTRODUCTION

Heretofore but slight attention has been paid to the germination of camphor seed. The few statements on this subject which occur in the literature refer only to the percentage of seeds germinating under conditions existing at the place of experimentation, and all the recorded results indicate a uniformly low germination. Likewise in Florida, previous to the experiments recorded in this article, the germination of camphor seed has been extremely low.

In commercial plantings in Florida, in which unpulped seeds have been planted with a modified cotton-dropping machine, the average number of seedlings brought to transplanting age on 1 acre of seed bed has been approximately 20,000. To plant an acre of seed bed requires 3 bushels of camphor seed, or approximately 200,000 seeds. The germination on a commercial scale, therefore, has averaged only about 10 per cent, which corresponds closely with the results obtained in various foreign countries. As a consequence of this low germination there has been no considerable extension of large plantings because of the limited number of seedlings available each year.

EXPERIMENTS IN 1916–17

In the fall of 1916 it was decided to make germination tests of camphor seed to determine if possible the cause or causes of the low germination obtained both experimentally and commercially. Accordingly seed was gathered from six individual trees growing in the vicinity of Orlando, Fla.

Seed from one parent tree, A, was selected from a row grown for shade and ornamental purposes. This tree was 20 years old and a typical representative of the camphor trees in Florida from which seed is gathered for commercial planting. The conditions under which the various lots of seed were collected and the treatment of each before planting are shown in Table I.

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TABLE I.—*Condition and treatment of camphor seed selected for germination tests conducted in 1916–17*

PARENT TREE A

Experimental row No.	Treatment of seed just previous to planting.
6	As they came from the parent tree.
7	Pulp removed.
10 North half	Pulp removed. Soaked in water at 25°C. for ½ hour.
10 South half	Pulp removed. Soaked in water at 50°C. for ½ hour.
13	Picked up from the ground. Pulp removed.
14	Picked up from the ground as they fell from the parent tree.
15	As they came from the parent tree. Gathered after a severe freeze.
16	Pulp removed. Gathered after a severe freeze.
17 North half	As they came from the parent tree. Gathered after a severe freeze and soaked in water for 18 hours.
17 South half	Pulp removed. Gathered after a severe freeze and soaked in water for 18 hours.

The first experiments were conducted in the winter of 1916–17. From some previous experience it was found that by removing the pulp from around the seed, germination was hastened if not materially increased. It was decided, therefore, to give special attention to the effect of removal of the pulp from the seed, since if it proved to be a decided aid to germination, the adoption of this method of treatment by commercial growers would be of distinct advantage. The remainder of the seed from the selected trees was pulped and planted. The percentage of germination was high, but the results are not recorded here since no data were secured on unpulped seed from the same trees.

The seed bed had been well prepared one week previous to the planting of the first seed, and a quantity of dry velvet-bean vines had been turned under. Drills from 1½ to 2 inches deep were opened with a hoe and the seed carefully hand-planted at intervals of 2 inches. The soil was placed back in the drill and very firmly packed. At the time of planting the soil was moist and in good condition, but later in the spring after the seedlings were several inches high it became necessary to water the bed three times in order to maintain the moisture content. On May 7 and August 1, 1917, the seed bed was fertilized with goat manure analyzing: Moisture 20 per cent, ammonia 1.5 per cent, and potash (as K_2O) 2.5 per cent. One hundred pounds were used at each application, which was at the rate of 1 ton per acre. The seedlings were well cared for by cultivating and hoeing. Table II gives the date of planting, rate of germination, and total percentage of germination in the 1916–17 trial of seed from parent tree A.

TABLE II.—*Rate and percentage of germination of camphor seed in the experiment of 1916–17*

PARENT TREE A

Experimental row No.	Date seed was gathered.	Date seed was planted.	Number of seeds planted.	Date first plants observed above ground.	Rapidity and percentage of germination.												Total germination.
					1917.										1918.[a]		
					Feb. 20.		Mar. 20.		Apr. 1.		Apr. 15.		May 10.		Jan. 4.		
	1916.			1917.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	Per cent.
6	Nov. 25	Dec. 1	1,000	Feb. 20	8	0.8	51	5.1	81	8.1	85	8.5	94	9.4	92	9.2	9.4
7	...do...	Dec. 2	1,000	Jan. 23 [b]	171	17.1	550	55.0	556	55.6	543	54.3	522	52.2	468	46.8	60.1
10 North half	...do...	...do...	250	Jan. 23 [c]	32	12.8	131	52.4	131	52.4	127	50.8	121	48.4	111	44.4	60.4
10 South half	...do...	...do...	250	Jan. 23 [d]	14	5.6	118	47.2	118	47.2	113	45.2	104	41.6	98	39.2	61.2
	1917.			1917.													
13	Jan. 3	Jan. 6	940	Mar. 12	.....	.....	91	9.6	147	15.6	140	14.8	132	14.0	122	13.0	15.6
14	...do...	...do...	1,000	Mar. 20	.....	.....	11	1.1	55	5.5	59	5.9	56	5.6	51	5.1	5.9
15	Feb. 6	Feb. 10	1,000	Apr. 1	.....	.....	.....	.....	2	.2	22	2.2	45	4.5	45	4.5	4.5
16	...do...	...do...	1,000	Mar. 12	.....	.....	55	5.5	326	32.6	316	31.6	302	30.2	285	28.5	32.6
17 North half	...do...	...do...	500	Apr. 1	.....	.....	.....	.....	3	.6	8	1.6	13	2.6	17	3.4	3.4
17 South half	...do...	...do...	500	Mar. 9	.....	.....	56	11.2	176	35.2	196	39.2	176	35.2	164	32.8	39.2

[a] Transplanting time.
[b] Forty-five plants frozen off Feb. 2, 1917, taken into calculation in determining maximum germination.
[c] Twenty plants frozen off Feb. 2, 1917, taken into calculation in determining maximum germination.
[d] Thirty-five plants frozen off Feb. 2, 1917, taken into calculation in determining maximum germination.

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No germination was secured when the seeds were artificially dried at a temperature of approximately 55° C. Seeds that were allowed to air-dry for several weeks in an attic likewise failed to germinate. Soaking in water at a temperature as high as 50° neither hastened nor impaired germination. Pulped seed treated with sulphuric acid of 5 per cent concentration by weight failed to germinate.

At the commercial planting beds so much seed is received in a fermenting condition that it was deemed advisable to ferment one lot of seed during an extended period in order to ascertain the effect on their germinating power. One thousand seeds fermented for 35 days in a closed jar failed to show a single case of germination. At the end of this period the pulp surrounding the seeds had almost entirely decomposed and the resulting liquid was sufficient to cover practically all the seeds. Shipments of seed for commercial use, however, are seldom enroute longer than from 8 to 10 days and do not reach such an advanced stage of decomposition. No marked ill effects due to fermentation have been noted in the commercial seed beds, which is attributed to the fact that the seed pulp has not entirely decomposed and that the liquid is constantly leaching from the barrels and boxes in which the seed is shipped, thus eliminating any chance for the seed to soak. Moreover, as soon as the seeds reach the camphor plantation they are spread out to cool and dry and fermentation ceases.

The results obtained from seed picked up from the ground are of special interest, such seed being often used in commercial work. Camphor seeds even when quite ripe do not drop readily from the tree; and a large percentage of the seeds which fall early in the season are defective, since the fallen unpulped seed showed a germination of only 5.9 per cent as compared with 9.4 per cent germination of seed picked from the tree. However, these defective seeds when pulped showed a germination of 15.6 per cent as compared with 60.1 per cent of pulped seed picked from the same tree. The seeds picked up from the ground were planted one month later than those picked from the tree, but they had fallen during the interim.

The idea that frozen camphor seed will not germinate is widely disseminated throughout Florida. A special experiment was made with seeds obtained after a relatively hard freeze during which the temperature fell to 26° F. The results obtained prove beyond doubt that camphor seed subjected to a freeze will germinate (fig. 1). This fact is of special value since freezing weather is liable to occur at any time during the late fall months in the camphor-seed producing areas, especially in those farthest north. The total germination, however, is decreased, being approximately 50 per cent of that obtained with unfrosted seeds. A greatly increased germination of the seed is secured by removing the pulp before planting. This increase was found to amount to 539 per cent. A graphic representation of the increased germination is presented in

figure 1, which shows not only the increased germination when the pulp is removed but the variation in germination of seed secured under varying conditions. The seed planted on December 1, 1916, which was picked from parent tree A, was first-class in every respect. On January 6, 1917, a quantity of seed was planted that was picked up from the ground under the same tree, and on February 6 frozen seed from this tree was secured and planted. In every instance the removal of the pulp before planting greatly increased the germination.

The percentage of germination of the seed picked up from the ground is much less than that of seed picked from the tree (fig. 1). This difference in germination was anticipated and was due in great part at least to defective seeds which fell from the tree, in other words, those which are considered as "drops." However, even the germination of these "drops" increased by 16.4 per cent when the pulp was removed. Seed picked from the tree after a severe freeze germinated remarkably well, especially when the pulp was removed. By soaking these frozen seeds in water at a temperature of approximately 25° C. for 18 hours a rather remarkable result was obtained. The

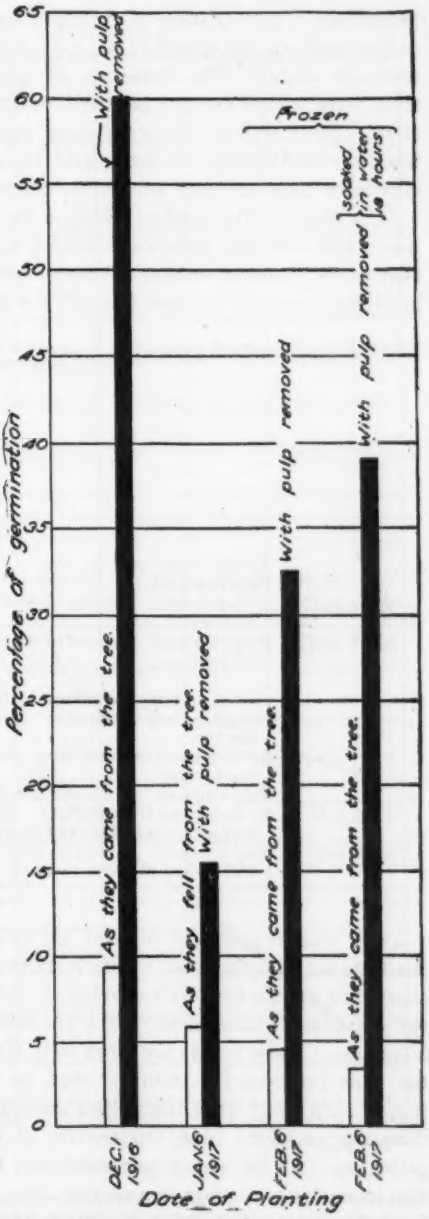


FIG. 1.—Diagram showing percentage of germination of camphor seed secured from parent tree A under varying conditions.

percentage of germination of unpulped seed was reduced, whereas that of pulped seed was increased 23 per cent over the germination of the pulped seed not soaked. The reduction in germination when the unpulped seeds were soaked can not be attributed to the direct action of the water alone. Since soaking favors a more rapid decomposition of the pulp when the seed is placed in the ground, the degree of fermentation reached may have been sufficient to destroy the vitality of some of the seeds.

In addition to the marked effect on the rapidity of germination which was noted when the pulp was removed from the seed, it was also noted that as the planting season advanced germination was more rapid and much less time was required to reach the point of maximum germination.

TABLE III.—*Rapidity of germination of camphor seed secured from parent tree A under various conditions*

Experimental row No.	Treatment of seed.	Date of planting.	Days required to reach maximum germination.
		1916.	
6	As they came from the tree	Dec. 1	161
7	Pulp removed	Dec. 2	120
10 North half	Pulp removed. Soaked in water at 25° C. for ½ hour	...do...	109
10 South half	Pulp removed. Soaked in water at 50° C. for ½ hour	...do...	109
		1917.	
13	Picked up from the ground. Pulp removed	Jan. 6	84
14	Picked up from the ground. As they fell from the tree	...do...	98
15	As they came from the tree. Gathered after hard freeze	Feb. 10	89
16	Pulp removed. Gathered after hard freeze	...do...	49
17 North half	As they came from the tree. Gathered after severe freeze and soaked in water 18 hours	...do...	89
17 South half	Pulp removed. Gathered after severe freeze and soaked in water 18 hours	...do...	64

Three special points of interest are brought out in Table III: First, the time for camphor seed to reach maximum germination; second, the shortening of this time by removing the pulp before planting; and third, the decrease in time required for the seed to germinate as the season advances. These points are more fully illustrated in the graph showing the time required for camphor seed to reach maximum germination (fig. 2). In every trial the pulped seed germinated much more quickly than the unpulped seed, irrespective of the condition at the time of gathering. As the season advanced and the soil warmed up, germination naturally took place in a shorter time. But what is of more interest from the commercial point of view is the fact that seed gathered and planted early in the fall will remain in the ground in good condition

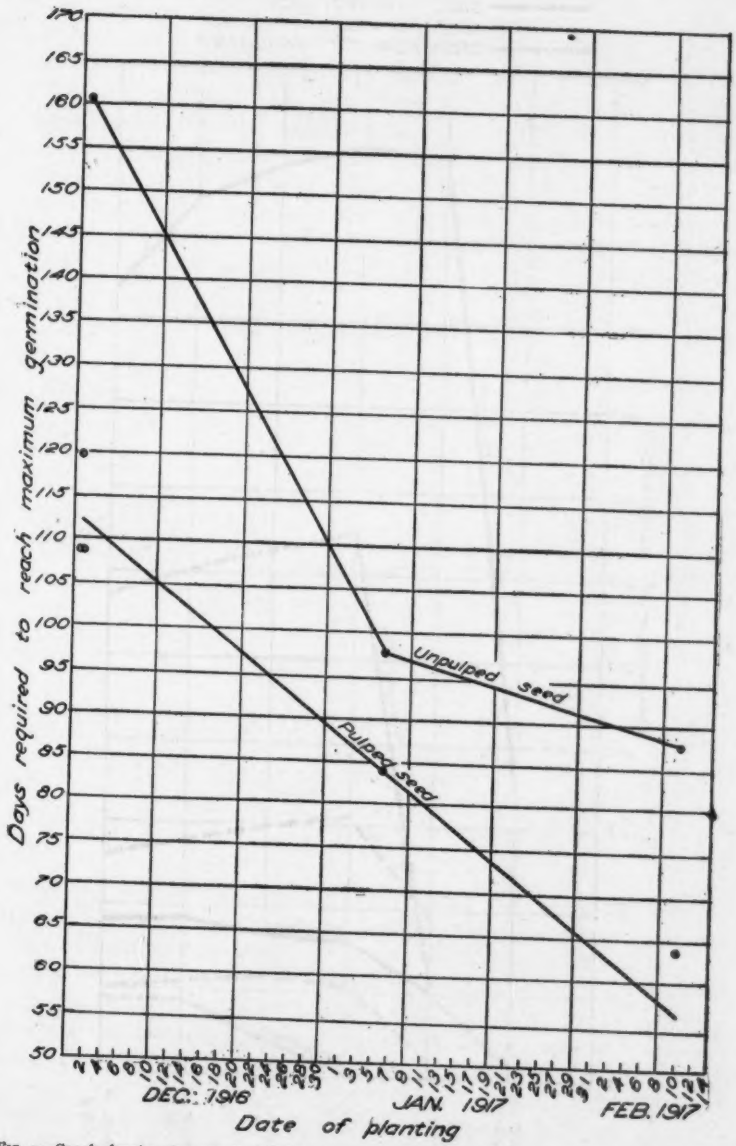


FIG. 2.—Graph showing time required for pulped and unpulped camphor seed to reach maximum germination. The seeds were planted at intervals of approximately one month during the winter of 1916–17.

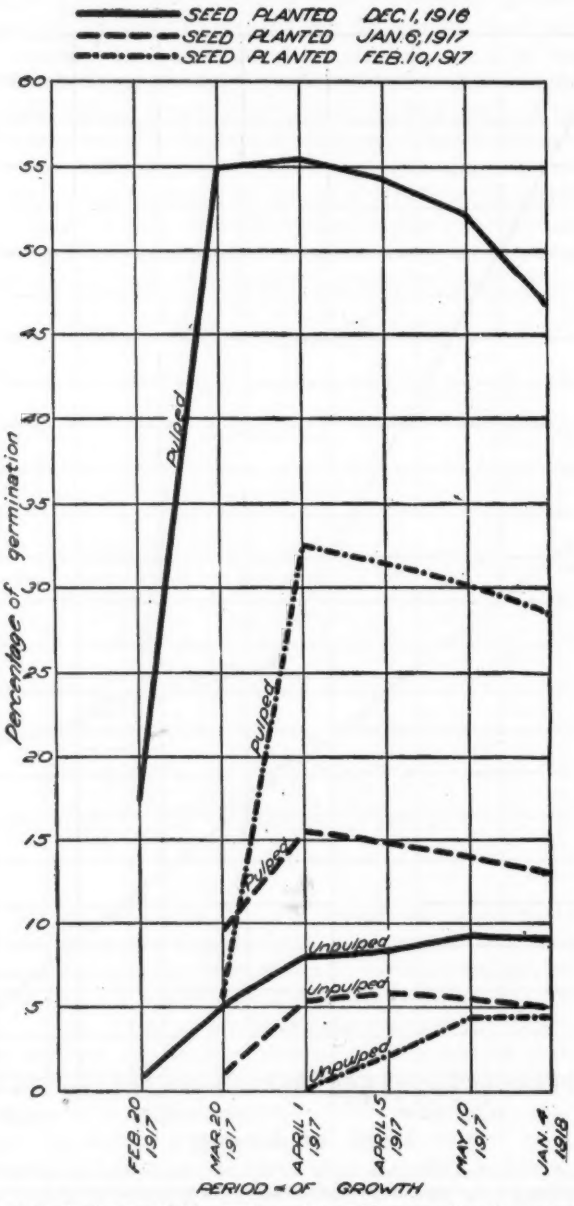


FIG. 3.—Graph showing time required for camphor seed secured from parent tree A at various times and under various conditions to reach maximum germination. The percentage of germination is also shown.

until favorable germinating weather is reached. Figure 3 shows graphically the time required to reach maximum germination and the percentage of germination of seed secured from parent tree A at various times and under various conditions. The maximum germination of all pulped seed was reached by April 1, 1917, although the time of planting extended over the period from December 2, 1916, to February 10, 1917; whereas the maximum germination of the unpulped seed occurred about May 10, 1917, the planting period extending over the same period of time as that of the pulped seed.

The results of the germination experiments of 1916–17 were so pronounced in favor of removal of the pulp from the camphor seed that the work for 1917–18 was planned to include a more extended comparison between pulped and unpulped seed.

EXPERIMENTS IN 1917–18

During the first week of November, 1917, a seed bed was prepared on soil practically identical with that employed in the experiments of 1916–17. A quantity of fertilizer made by composting rose-geranium leaves and stalks—after distilling the volatile oil—was turned under at the time the seed bed was plowed. This fertilizer material consisted only of leaves and stalks and, being somewhat intact at the time of application, had a tendency to keep the soil from packing, at the same time supplying some plant food as it decomposed. At the time of planting the soil was moist, and no subsequent watering of the bed was necessary throughout the time of the experiments. No fertilizer was applied during the growing season. The plants were given the usual cultivation and hoeing. Commercial conditions, with the exception of the application of fertilizer, were approximated as closely as possible.

Seed was selected from a row of ornamental camphor trees, and trees were chosen which bore an abundance of fruit. Camphor seed which ripens on the tree falls readily into the hand when picked. All the seeds used in these experiments were fully ripened and easily secured by picking, care being taken to secure seed from all sides of the tree. Each sample therefore was representative of the entire yield of the individual tree. All the seeds were gathered on November 27, 1917, and planted November 28, 1917. A severe freeze occurred February 2, 1918, but as none of the seedlings had appeared above ground no damage was done. In Table IV are given data in reference to the treatment of the seed, rapidity of germination, and percentage of total germination.

TABLE IV.—*Rate and percentage of germination of camphor seed in the experiments of 1917–18*

Parent tree.	No. of row.	Treatment of seeds planted.	Number of seeds planted.	Rapidity and percentage of germination. 1918. Feb. 11. No.	Feb. 11. Per ct.	Feb. 23. No.	Feb. 23. Per ct.	Mar. 18. No.	Mar. 18. Per ct.	Apr. 27. No.	Apr. 27. Per ct.	July 6. No.	July 6. Per ct.	Dec. 30. No.	Dec. 30. Per ct.	Total germination. Per ct.
A	1 North half	None	300	....	....	2	0.6	2	0.6	18	3.0	18	3.0	18	3.0	3.0
	1 South half	Pulp removed	300	48	16.0	199	66.0	223	74.0	232	77.0	232	77.0	232	77.0	77.0
B	2 North half	Pulp removed	375	137	36.5	311	82.9	325	86.6	317	84.5	317	84.5	317	84.5	86.6
	2 South half	None	500	....	....	15	3.0	67	13.4	117	23.4	101	20.2	101	20.2	23.4
C	3 North half	None	500	....	....	11	2.2	22	4.4	55	11.0	55	11.0	55	11.0	11.0
	3 South half	Pulp removed	500	49	9.8	345	69.0	375	75.0	315	63.0	259	51.8	259	51.8	75.0
D	4 North half	Pulp removed	500	181	36.2	362	72.4	367	73.4	367	73.4	377	75.4	377	75.4	75.4
	4 South half	None	500	....	....	15	3.0	40	8.0	80	16.0	69	13.8	69	13.8	16.0
E	5 North half	None	500	....	....	3	.6	9	1.8	80	16.0	63	12.6	63	12.6	16.0
	5 South half	Pulp removed	500	65	13.0	218	43.6	314	62.9	326	65.2	310	62.0	310	62.0	65.2
G	6 North half	Pulp removed	500	273	54.6	372	74.4	388	77.6	382	76.4	442	88.4	442	88.4	89.6
	6 South half	None	500	....	....	15	3.0	42	8.2	81	16.2	63	12.2	63	12.2	16.2
H	7 North half	None	500	....	....	3	.6	4	.8	35	7.0	33	6.6	33	6.6	7.0
	7 South half	Pulp removed	500	142	28.4	301	60.2	360	72.0	362	72.4	373	74.6	373	74.6	74.6
K	8 North half	Pulp removed	500	157	31.4	315	63.0	336	67.2	316	63.2	349	69.9	349	69.8	73.8
	8 South half	None	500	....	....	2	.4	11	2.2	43	8.6	31	5.2	31	5.2	8.6
O	9 North half	None	500	....	....	7	1.4	29	5.8	43	8.6	37	7.4	37	7.4	8.6
	9 South half	Pulp removed	500	114	22.8	332	66.4	397	79.4	375	75.0	382	76.4	382	76.4	80.8
P	10 North half	Pulp removed	500	151	30.2	394	78.8	409	81.8	352	70.4	418	83.6	418	83.6	95.4
	10 South half	None	500	....	....	8	1.6	31	6.2	40	8.0	36	7.2	36	7.2	8.0

In this trial the seeds from a total of 10 individual trees were tested. The results obtained by merely pulping the seed before planting were so favorable that commercial planters adopted the pulping plan when its merits were brought to their attention. In commercial work the pulp is removed by rubbing the seeds through a wire screen of the proper mesh. Many of the pulps are left behind and are swept off the screen. Those that fall through with the seed cause no inconvenience in planting, for the seed is spread to dry for about 24 to 48 hours; and during this time the pulps dry and shrink to such an extent that they readily pass through the plates of the corn planter which is now used to plant the pulped seed. Unless the seed is dried before planting the plates of the planter become clogged, causing an uneven distribution of the seeds in the row.

TOTAL GERMINATION OF CAMPHOR SEED

The greatly increased germination obtained when the seed is pulped is remarkable. Figure 4 shows graphically the total germination of both the pulped and unpulped seed from 10 parent trees. The increased germination of the pulped over the unpulped seed ranged from 270 per cent for tree B to 2,466 per cent for tree A, the average increase for the entire lot of 10 trees being approximately 525 per cent.

Germination was found to be uneven with seed from various parent trees. Moreover, the ratio between the percentage of germination of the unpulped and the pulped seed was by no means constant; and no correlation can be established between the percentage of germination

when the seed is pulped and when it is not. The variation in germination of 10 individual lots of seeds from as many parent trees is shown graphically in figure 5. The upper line in each case indicates the germination of pulped seed, the lower line the germination of unpulped seed. Under period of growth, *a* indicates the date of planting, November 28, 1917. The percentage of seeds germinating was determined by counting the number of seedlings in the beds at stated times, indicated in the figure as follows: *b*=February 11, 1918; *c*=February 23, 1918; *d*=

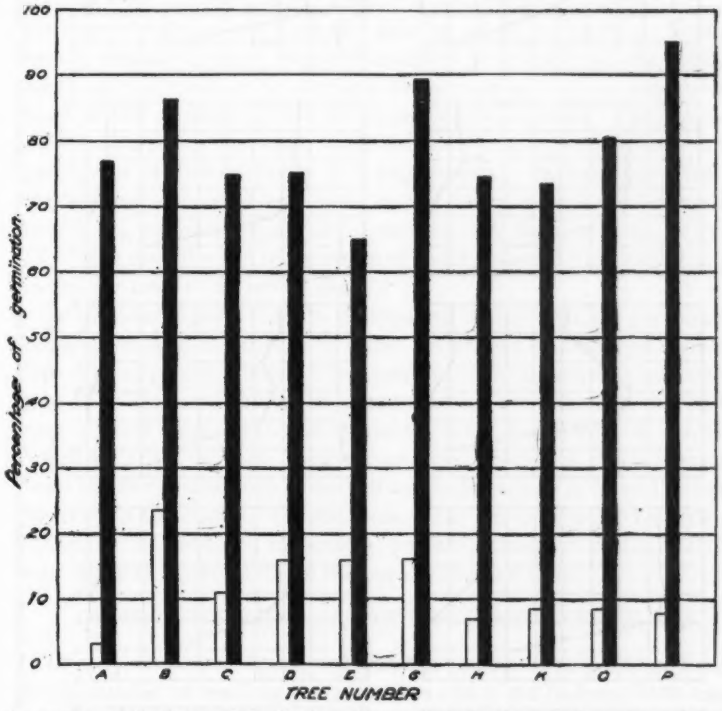


FIG. 4.—Diagram showing percentage of total germination of pulped and unpulped camphor seed from 10 parent trees. Black bars represent pulped seed; white bars, unpulped seed.

March 18, 1918; *e*=April 27, 1918; *f*=July 6, 1918; *g*=December 1, 1918, on which date the seedlings were transplanted. It will be noted that the pulped seed germinated in much shorter time than the unpulped seed and that after the appearance of the first seedlings the major portion of the germination took place in a relatively short time. The apparent falling off in the germination of the pulped seed, as indicated in the graphs (fig. 5), is due to the effect of the hot sun on the tender seedlings. A large number of the seedlings were burned off at the ground level soon after they pushed up through the hot sand, and

as a result many of those represented in the count of one day had disappeared by the time of the next count. Likewise some of the seed that germinated never entered into the calculations, the seedlings being lost to observation between counts.

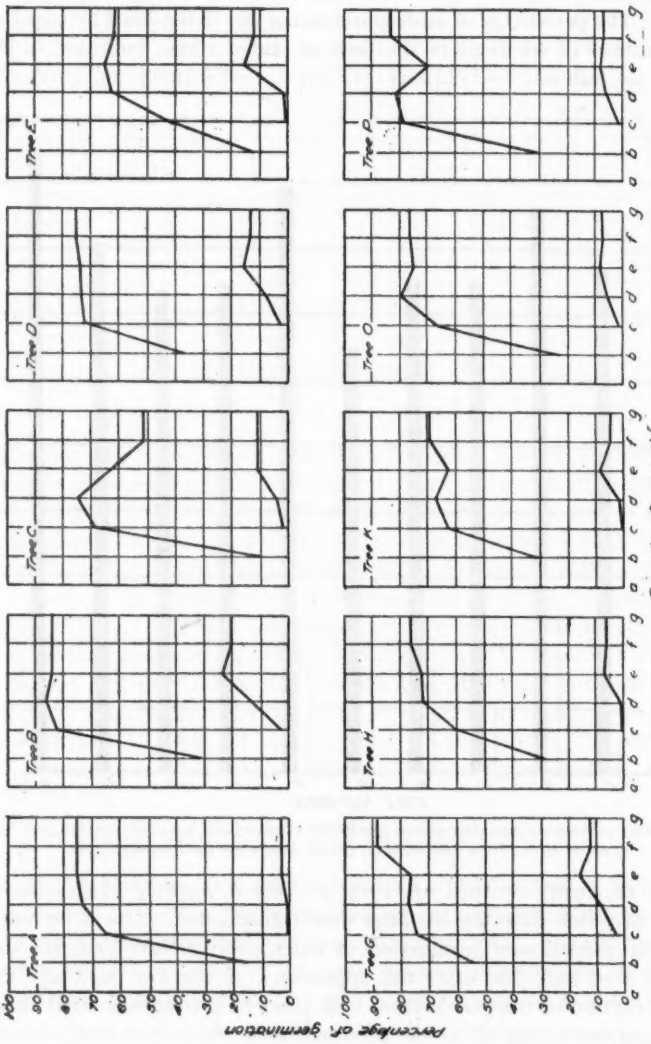


FIG. 5.—Graphs showing rates and percentage of germination of pulp and unpulped camphor seed from 10 parent trees. For explanation, see text.

Two of the parent trees, A and D, show no apparent falling off in the curve of germination for pulped seed; and parent trees A and C show likewise no apparent falling off for unpulped seed. Careful observation

has shown that the burning off of the young seedlings occurred to a greater or less degree throughout all the late winter and early spring and that the burning off was by no means uniform in all the experimental rows. It appears, therefore, that the curves given in figure 5 are not the true curves of germination, but rather the curves of count. However, the true curve of germination follows closely the curve of count with some striking exceptions. As the season advanced a large number of seeds germinated within a short period of time, and as the heat of the sun became more intense a large number of seedlings were burned off. In the case of eight of the trees the burning off of the seedlings between counts reached a point where it included practically all the younger seedlings just pushing up through the ground as well as some of the more tender seedlings of the count previously recorded. Consequently a drop occurred in the curve, which shows as an apparent decrease in the percentage of germination. This explanation is further supported by the fact that somewhat later in the spring, during more favorable weather conditions, germination of more of the seed took place, as shown in Table IV, which caused a rise in the curve of count, especially noticeable in the curves for trees G, K, and P (fig. 5).

In the case of parent tree A, no apparent falling off in the germination of camphor seed is recorded in either the pulped or unpulped seeds; in tree C no apparent falling off is recorded for the unpulped seed; and in tree D no apparent falling off is recorded for the pulped seed. In the case of these trees, A, C, and D, the burning off of the young seedlings which occurred between observations never reached a stage where the total number of seedlings burned off was large enough to cause a decrease to show in the count. For this reason the curve of count for trees A, C, and D, as indicated in figure 5, probably closely coincides with the true curve of germination for the seed from these trees.

CAMPHOR SEEDLINGS BROUGHT TO TRANSPLANTING SIZE

Of more economic importance than the number of seed that germinate is the number of seedling camphor trees which can be brought to transplanting size. Out of 4,800 seeds planted as they came from the tree only 508 seedlings reached a sufficient size for transplanting, whereas from 4,675 seed planted after pulping 3,499 such seedlings were secured. The increase therefore in the percentage of seedlings of transplanting size from the pulped seed over those from the unpulped seed amounted to approximately 600 per cent.

The loss of seedlings due to the burning off by the hot sun is relatively large and has a marked influence on the percentage of seedlings secured. In this experiment 14.5 per cent of the total number of seedlings obtained from the unpulped seed and 5.5 per cent of the seedlings from the pulped seed were burned off. However, the total loss of seedlings from pulped

seed is much less than from unpulped seed, and this lower percentage is due to the advanced growth obtained by the seedlings before the extreme hot weather commenced.

In Plate 20 is shown a nursery bed of camphor seedlings just previous to transplanting on December 1, 1918. In this bed the pulped and the unpulped seed were planted in alternate rows. The small seedlings from the unpulped seed are almost obscured by the alternate rows of large seedlings which were obtained from the pulped seed.

At the time of transplanting, the seedlings had reached the comparative size shown in Plate 21 A. In all instances the pulped seed had produced hardier and more vigorous seedlings, which, when trimmed and cut back as shown in Plate 21 B, were in a better condition to withstand the shock of transplanting. The superior growth of the seedlings from pulped seed was so marked that measurements were taken of 258 seedlings of this lot and compared with measurements taken of the same number of seedlings that came from unpulped seed. The results are given in Table V.

TABLE V.—*Average growth attained by camphor seedlings during the growing period between germination of the seed and transplanting of the seedlings*

Treatment of seed.	Number of seedlings measured.	Average growth.		
		Length of stem.	Length of taproot.	Diameter of crown.
		Inches.	*Inches.*	*Inches.*
Pulp removed	258	13.0	17.7	0.473
Pulp not removed	258	11.0	15.3	.320

If the growth of seedlings from seed on which the pulp remained is considered as 100 per cent, then the increased growth in the seedlings from the pulped seed is: For length of stem, 18.1 per cent; for length of taproot, 15.6 per cent; and for diameter of crown, 47.8 per cent. The latter vigorous growth is of special interest from the commercial point of view, since the loss from transplanting is much less with roots of large diameter than it is with small roots, which are more easily dried out during the period that elapses between the removal of the seedlings from the seed bed and the date of the beginning of growth the following growing season. The increased growth of the seedlings is brought about directly by pulping the seed, since it insures a more rapid germination and gives the plant an early start in the spring and consequently a much longer growing season. The increased growth and vigor reduce to a very low figure the loss through transplanting.

SUMMARY AND CONCLUSIONS

In the season of 1916–17 camphor seeds were planted under various conditions. The experiment was repeated in the season of 1917–18, and commercial conditions were closely approximated.

Removing the pulp from the seed was found to hasten germination by an average of two weeks; it also gave an increase in germination of approximately 525 per cent over that of unpulped seed.

Drying the seed with artificial heat at 55° C. destroyed all vitality.

Soaking the seed in water apparently did not hasten germination; neither did it increase the percentage of seed that germinated.

Soaking the seed in sulphuric acid of 5 per cent by weight destroyed all vitality.

Allowing the seed to ferment and the pulp to decompose in a closed vessel destroyed all vitality.

Seed picked up from the ground showed less vitality than those picked from the tree, but removal of the pulp increased and hastened germination.

A freeze on three successive nights, during which the temperature fell to 26° F., did not destroy the vitality of all the seed; it did, however, impair the vitality and reduce the number of seeds that germinated by approximately 50 per cent.

Seeds planted early in the winter required a longer time to germinate than those planted in midwinter. The former averaged more sturdy trees.

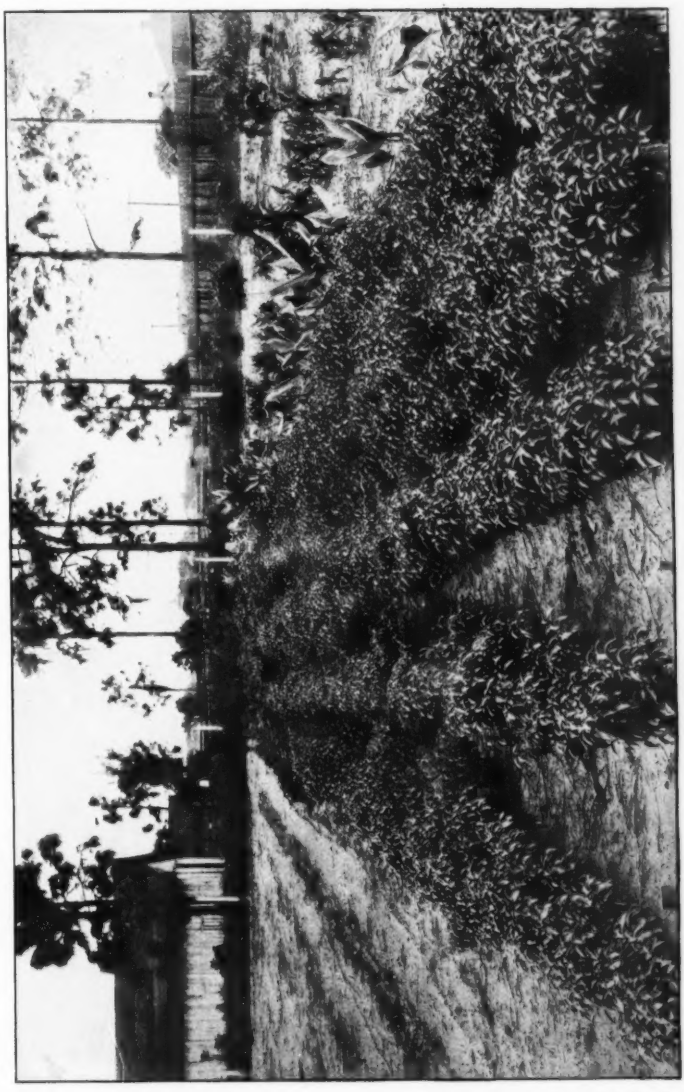
When the pulp was removed and the germination of the seed thus hastened, a larger and more sturdy seedling tree was obtained for transplanting than when the pulp was not removed. The increase in the number of seedlings of transplanting size secured by pulping the seed amounted approximately to 600 per cent.

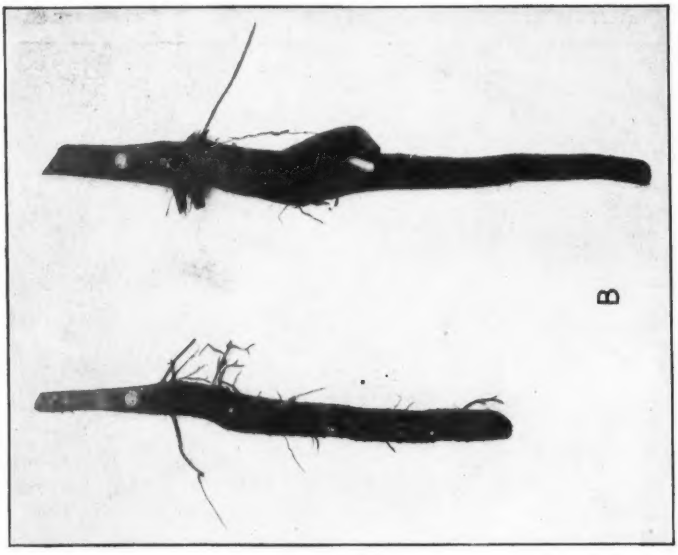
From a commercial point of view, removal of the pulp is desirable even though the labor must all be done by hand. The increased germination and the well-developed trees that result will repay many times the cost of the labor involved.

It is believed that in commercial plantings the removal of the pulp from the seeds will increase the percentage of germination by at least 200 per cent, thus producing 40,000 more seedling trees to each acre of seed bed. This estimate is believed to be very conservative, and even a much greater increase may be expected.

PLATE 20

A camphor seed bed, showing the growth of seedlings from pulped and unpulped camphor seed planted in alternate rows. The seedlings growing from pulped seed have been cut away on one side in order to expose the seedlings growing from unpulped seed, which are otherwise almost completely covered by the luxuriant growth of the former.

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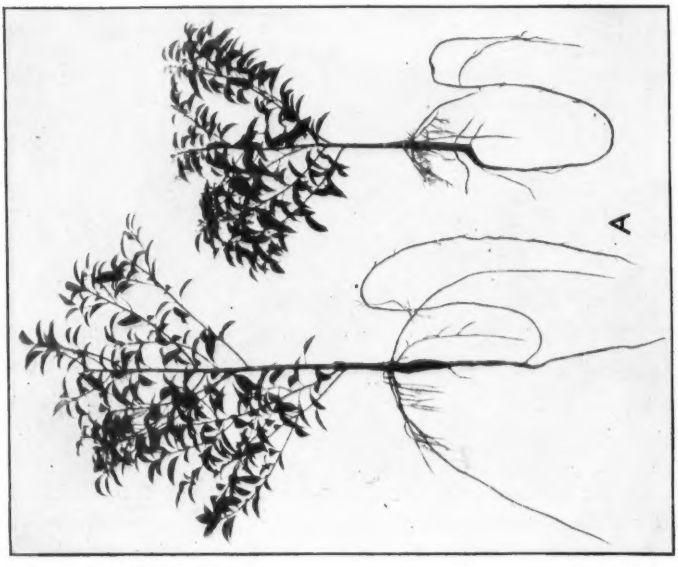
A

PLATE 21

A.—Camphor seedlings at the time of transplanting. The tree on the left is a representative produced from pulped camphor seed; the one on the right is a representative produced from unpulped camphor seed. Both seedlings are from seed of the same parent tree and both are of the same age from planting of the seed.

B.—Camphor seedlings cut back and trimmed ready for transplanting. These seedlings are the same as those shown in A. The one on the right is from pulped seed.

BACTERIUM ABORTUS INFECTION OF BULLS

[PRELIMINARY REPORT]

By J. M. Buck, G. T. Creech, and H. H. Ladson, *Pathological Division, Bureau of Animal Industry, United States Department of Agriculture*

Numerous investigators have called attention to the fact that *Bacterium abortus* agglutinins and complement-fixing bodies can frequently be demonstrated in the blood serum of bulls from abortion-infected herds. Such animals in consequence have frequently been referred to as being systemically infected. While the presence of these bodies constitutes strong evidence that abortion infection exists, or has been present, success has been reported in associating positive reactions with the causative infection in so few instances as to have resulted in a certain amount of speculation regarding the significance of these reactions in male animals.

Literature, it is true, records no great amount of investigative work in connection with bulls suspected of being infected with abortion disease where the object has been the isolation of the causative microorganism from the organs or tissues of the animals or the demonstration of lesions associated therewith.

Schroeder and Cotton [1] in investigating this problem describe two cases that came under their observation. They state that one of the bulls at the time of autopsy showed the presence of an abscess involving the epididymis of one testicle from which *Bact. abortus* was isolated. The other animal was permitted to serve a cow that was considered to be free from abortion disease. Seminal fluid which was recovered from the vagina immediately following the service and injected into numerous guinea pigs produced *Bact. abortus* lesions in one of the experimental animals.

Rettger and White [2] describe endeavors to associate the presence of the infection with positive serum reactions in three cases which they studied. In two of the animals neither abortus infection nor pathological changes could be demonstrated. In the third they call attention to the finding of two abscesses or cysts in the region of the groin, near the point of attachment of the scrotum; but from these abscesses they were unable to isolate the abortion organism, thus failing to obtain bacteriological evidence of the infection.

In view of the positive bacteriological findings of Schroeder and Cotton the present writers were prompted to undertake further investigations,

[1] Schroeder, E. C., and Cotton, W. E. Some facts about abortion disease. *In* Jour. Agr. Research, v. 9, no. 1, p. 9–16. 1917.

[2] Rettger, L. F., and White, G. C. Infectious abortion in cattle. Conn. Storrs Agr. Exp. Sta. Bul. 93, p. 199–249. 1918. References, p. 246.

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involving a considerable number of animals, in an endeavor to ascertain with what frequency abortus infection could be demonstrated in the generative organs of bulls giving positive or suspicious reactions to the agglutination test for this disease, and to determine whether or not pathological changes are commonly associated with such infection.

EXPERIMENTAL PROCEDURE

The procedure employed by the writers consisted in securing blood samples from the animals as they arrived at one of the abattoirs in close proximity to Washington, D. C., for slaughter. No information was available regarding the original source of the bulls or the exposure sustained. Each sample was given a number corresponding to the serial number of a tag that was attached to the animal's ear at the time of bleeding. The blood samples were then taken to the laboratory for the application of the agglutination test. At the time of slaughter, which was usually the following day, those animals giving positive or suspicious reactions were autopsied as carefully as abattoir conditions permitted and the organs of the genital system were secured for further study. Cultural work was depended upon as a means of detecting infection, the medium employed consisting of 3 per cent glycerin infusion agar to which approximately 5 per cent sterile blood serum was added. To reduce the oxygen tension the inoculated tubes were subjected to incubator temperature in closed jars in the presence of fresh cultures of *Bacillus subtilis*.

During the period from December 9, 1916, to July 7, 1918, the agglutination test for abortion disease was applied to 325 mature bulls. Of this number 288 gave negative results to the test. The manner in which the remaining 37 reacted is of considerable interest, inasmuch as the intensity of the reactions appeared to bear some relation to the cultural results.

The manner of applying the test consisted in the making of a 1 to 10 basic dilution of the blood serum. To the four tubes utilized for each case were added 0.4, 0.2, 0.1 and 0.05 cc. of this basic dilution. The amount of test fluid added to each tube was 1 cc.

The vesiculae seminales, vasa deferentia, testes, and epididymides were secured from the 37 bulls whose blood serum showed the presence of *Bact. abortus* agglutinins. From 15 to 20 tubes of medium were utilized for culturing the various organs from each bull. These investigations resulted in the demonstration of the presence of *Bact. abortus* infection in four animals—No. 88, 98, 136, and 409—and in the detection of marked lesions in bulls 98 and 409.

A brief description of the work performed and the findings in these cases follow.

The agglutination reactions of the animals appear in the following table.

TABLE I.—*Results of agglutination tests*

Animal No.	Suspected serum.				Animal No.	Suspected serum.			
	0.04 cc.	0.02 cc.	0.01 cc.	0.005 cc.		0.04 cc.	0.02 cc.	0.01 cc.	0.005 cc.
21	Sl	Sl	−	−	168	+	Sl	Sl	−
84	+	Sl	−	−	177	Sl	−	−	−
86	Sl	Sl	−	−	178	+	Sl	−	−
88	+	+	+	Sl	179	Sl	Sl	−	−
89	Sl	Sl	−	−	189	+	Sl	−	−
98	+	+	+	Sl	198	+	+	Sl	−
103	+	+	+	+	265	+	Sl	−	−
105	+	Sl	−	−	271	+	+	Sl	−
109	+	Sl	−	−	280	+	Sl	−	−
133	+	+	+	+	301	Sl	Sl	−	−
134	Sl	Sl	Sl	−	319	+	Sl	−	−
136	+	+	+	Sl	326	+	+	Sl	−
137	+	+	Sl	Sl	338	Sl	Sl	−	−
143	Sl	−	−	−	348	Sl	−	−	−
146	+	Sl	−	−	409	+	+	+	+
147	Sl	−	−	−	451	+	+	+	−
150	+	+	Sl	−	453	+	Sl	Sl	−
154	Sl	Sl	−	−	454	+	+	Sl	−
165	Sl	Sl	−	−					

+=Complete agglutination.
−=No agglutination.
Sl=Partial clumping of bacteria.

EXAMINATION AND FINDINGS OF BULL 88

February 12, 1918. Agglutination test: 0.04 cc.+, 0.02 cc.+, 0.01 cc.+, 0.005 cc. Sl.

February 14, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—Fluid of left seminal vesicle turbid in appearance and slightly more excessive in amount than that contained by other organ. No indication of abnormal conditions noted elsewhere.

BACTERIOLOGICAL FINDINGS.—Of the 16 tubes of medium inoculated from the various organs enumerated, after four days' incubation three tubes developed from 40 to 60 colonies of an organism suggestive of *Bact. abortus* and subsequently identified as such. These inoculations were from the left seminal vesicle.

EXAMINATION AND FINDINGS OF BULL 98

March 8, 1918. Agglutination test: 0.04 cc.+, 0.02 cc.+, 0.01 cc.+, 0.005 cc. Sl.

March 9, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—Marked pathological changes involved the left seminal vesicle. The organ was increased from 8 to 10 times

its normal size. On cross section of the vesicle numerous hemorrhagic areas were observed, as well as a number of necrotic centers, the latter being confined chiefly to the more central portions of the organ. So softened were some of these foci that the necrotic material assumed a semifluid character. The capsule of the organ showed considerable thickening. (See Pl. 22.)

HISTOLOGICAL EXAMINATION.—Sections from the left seminal vesicle showed varying stages of the diseased process, ranging from exfoliation of the epithelial lining of a few of the acini to complete obliteration of the normal glandular structure. There was marked proliferation of the interstitial tissue with round cell infiltration, which was more pronounced immediately surrounding the acini and just beneath the epithelial lining of the acini. In those areas exhibiting the more pronounced pathological changes many of the acini were filled with detached epithelial cells and cell débris. In other areas where the mere outline of the acini could be traced, a homogeneous substance was present, together with more or less granular detritus. There were hemorrhages into and between the acini. Occasionally large areas of degeneration and necrosis were observed. As a result of the inflammatory changes little normal glandular structure was recognized in many of the sections examined (Pl. 24, A and B). Plate 23 A, representing a normal seminal vesicle, is inserted for comparison with Plate 23 B, and Plate 24, A and B.

BACTERIOLOGICAL FINDINGS.—Eighteen tubes of medium were utilized for the culturing of the different organs. The six tubes from the left seminal vesicle after three days' incubation developed from 75 to 150 colonies of an organism that appeared typical of *Bact. abortus*. All tubes inoculated from other sources remained sterile, although incubated for several additional days. Subsequent work with the organism isolated established its identity as *Bact. abortus* and indicated that pure cultures of the organism were isolated in all instances.

EXAMINATION AND FINDINGS OF BULL 136

May 15, 1918. Agglutination test: 0.04 cc.+, 0.02 cc.+, 0.01 cc.+, 0.005 cc. Sl.

May 16, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—The right seminal vesicle showed slight enlargement. The fluid contained by this organ presented a turbid appearance. No lesions were elsewhere detected.

BACTERIOLOGICAL FINDINGS.—Five of the 18 tubes of medium inoculated from the different organs developed from 40 to 60 colonies of an organism that was subsequently identified as *Bact. abortus*. These inoculations were from the right seminal vesicle.

EXAMINATION AND FINDINGS OF BULL 409

August 15, 1918. Agglutination test: 0.04 cc.+, 0.02 cc.+, 0.01 cc.+, 0.005 cc.+.

August 16, 1918. Slaughtered.

MACROSCOPIC EXAMINATION.—Left seminal vesicle showed evidence of disease. This organ was approximately twice the size of the right and was incised with considerable difficulty on account of fibrous tissue proliferation. The fluid contained was decidedly turbid. Other organs presented a normal appearance.

MICROSCOPIC EXAMINATION.—Sections from the left seminal vesicle showed marked proliferation of the interstitial tissue with areas of round-cell infiltration. Degeneration and exfoliation of the epithelial cells lining the acini were observed. A few of the acini contained cells and cell detritus; others had been completely obliterated as a result of the inflammatory process. (See Pl. 23 B.)

BACTERIOLOGICAL FINDINGS.—Two of the 20 tubes of medium that were inoculated from the various organs developed colonies typical of *Bact. abortus*. The colonies were few in number and appeared on but 2 of 5 tubes that were sown with material from near the same point. These tubes were from the left seminal vesicle. All tubes inoculated from other sources remained sterile. The infection was subsequently established as *Bact. abortus*.

It has been previously suggested that the intensity of the serum reactions appeared to bear some relation to the cultural results. Of the 37 bulls exhibiting agglutinating properties for a *Bact. abortus* suspension, the blood serum of but 7 caused perfect agglutination of a suspension with 0.01 cc. of the serum. It may be observed that 4 of these 7 animals yielded positive cultural results and that in no instance was the presence of the infection demonstrated in animals when their blood serum failed to cause perfect agglutination with such an amount of serum.

EXAMINATION AND FINDINGS OF GUERNSEY BULL

Since the isolation of *Bact. abortus* infection from the cases previously described, the writers have had an opportunity to demonstrate the presence of the infection and observe lesions in a fifth bull where the isolation of abortion bacteria was carried out under different conditions and where it was possible to obtain a somewhat more complete history in regard to the development of the pathological changes that were associated with the infection.

This pure-bred Guernsey, 8 years of age, was acquired by the present owner in June, 1918, and appeared at the time to be in perfect physical condition.

The writers were informed that during the following January an asymmetrical enlargement of the scrotum was noted. Mechanical injury was

suspected which had prompted the application of fomentations and counterirritants. When the condition failed to respond to this treatment and an area of softening that appeared to involve the left testicle was later detected, a canula had been introduced through which had been evacuated a considerable quantity of a semifluid material. It was furthermore stated that the animal had at times discharged through the urethra a substance bearing some resemblance to that removed by the surgical procedure.

On April 22, 1919, about three months after the swelling was first observed, a sample of blood was secured for the application of the agglutination test for abortion disease. The specimen caused clumping of a *Bact. abortus* suspension with 0.0001 cc. of the serum.

When the animal was examined on the following day with the object of obtaining material for bacteriological work, the enlargement involving the left testicle was found to be four or five times the size of the normal organ. When a needle was passed into its lateral wall, little resistance was encountered after the instrument had been inserted for about 1½ inches. Through the needle were aspirated from 400 to 500 cc. of a gray-colored substance of the consistence of heavy cream. At the same time from 20 to 30 cc. of a turbid fluid were obtained from the urethral opening. This material was secured by exerting pressure on the urethra and by massaging the seminal vesicles through the walls of the rectum. During this procedure it was detected that the seminal vesicles differed markedly in size, enlargement of the right organ being pronounced.

CULTURAL RESULTS.—Eight tubes of serum agar were inoculated with the semifluid substance aspirated from the interior of the enlargement involving the diseased testicle. Numerous dilutions were made of the fluid recovered from the urethra with physiological salt solution, and serum-agar tubes were sown with these dilutions. When the tubes were examined after six days' incubation one colony of abortuslike appearance was observed on one of the tubes from the substance obtained by aspiration. The infection was later established as *Bact. abortus*. Further inoculations of medium with like material resulted in the isolation of additional abortus colonies, although fewer in number than were anticipated from the extent of the lesions. No *Bact. abortus* was isolated by cultural methods from the material secured from the urethra, but excessive contamination made these results inconclusive.

On May 9, or about two weeks after the condition was diagnosed as abortus infection, an opportunity was afforded for the making of a more thorough examination of the diseased process involving the external genitals and for further bacteriological work, for the affected testicle with its coverings were at this time removed and forwarded to the Pathological Division.

The weight of the mass of tissue was 5½ pounds. On section it was found to consist of an outer wall or capsule of from 1½ to 2 inches in thickness. This abnormal structure had evidently resulted from proliferative changes involving mainly the connective tissue coverings of the testicle. Firmly embedded in this external layer could be distinguished areas of tissue that upon microscopic examination were identified as epididymis that had undergone severe inflammatory changes. The cavity formed by this dense fibrous wall contained a considerable quantity of a grayish-colored, semifluid material identical with the substance previously obtained by aspiration. Floating free in the cavity was also a mass of tissue that was recognized as the remains of the testicle, it having the same general form although somewhat reduced in size. Blood vessels no longer communicated with the organ, and the serous membranes which normally envelop it had seemingly been entirely obliterated. The close resemblance existing between the semifluid substance and softened portions of the testicle strongly indicated that the organ was undergoing liquefaction necrosis.

MICROSCOPIC EXAMINATION.—The thick wall surrounding the testicle consisted largely of dense fibrous tissue with a certain amount of round-cell infiltration. Different portions of the epididymis which were embedded in this mass showed extensive interstitial proliferation, which had resulted in a wide separation of the tubules. Chronic inflammatory changes were noted in sections from the testicle proper. Many tubules were surrounded by zones of round-cell infiltration. There was exfoliation and more or less disintegration of the epithelium lining the tubules, causing the latter to be largely occupied by cell débris. Advanced degenerative changes, verging on necrosis, were observed in all the sections examined, the peripheral portion of the organ exhibiting little more than a mere outline of the testicle structure.

BACTERIOLOGICAL FINDINGS.—Tubes of serum agar that were inoculated with the exudate at this time developed numerous colonies of an organism that was identified as *Bact. abortus*.

It has been suggested by writers on abortion disease that *Bact. abortus* infection when acquired by bulls remains active for a comparatively brief period, the resistance offered being sufficient for its destruction. The encountering of a considerable number of animals giving slight agglutination reactions and the isolation of abortus infection from only a small percentage of the bulls cultured would tend to strengthen the theory that the infection may commonly terminate in this manner. On the other hand the extensive pathological changes and the chronic character of the lesions exhibited by three of the five bulls where abortus infection was demonstrated suggest that it may be unwise to assume that long-standing cases of infection never exist.

CONCLUSIONS

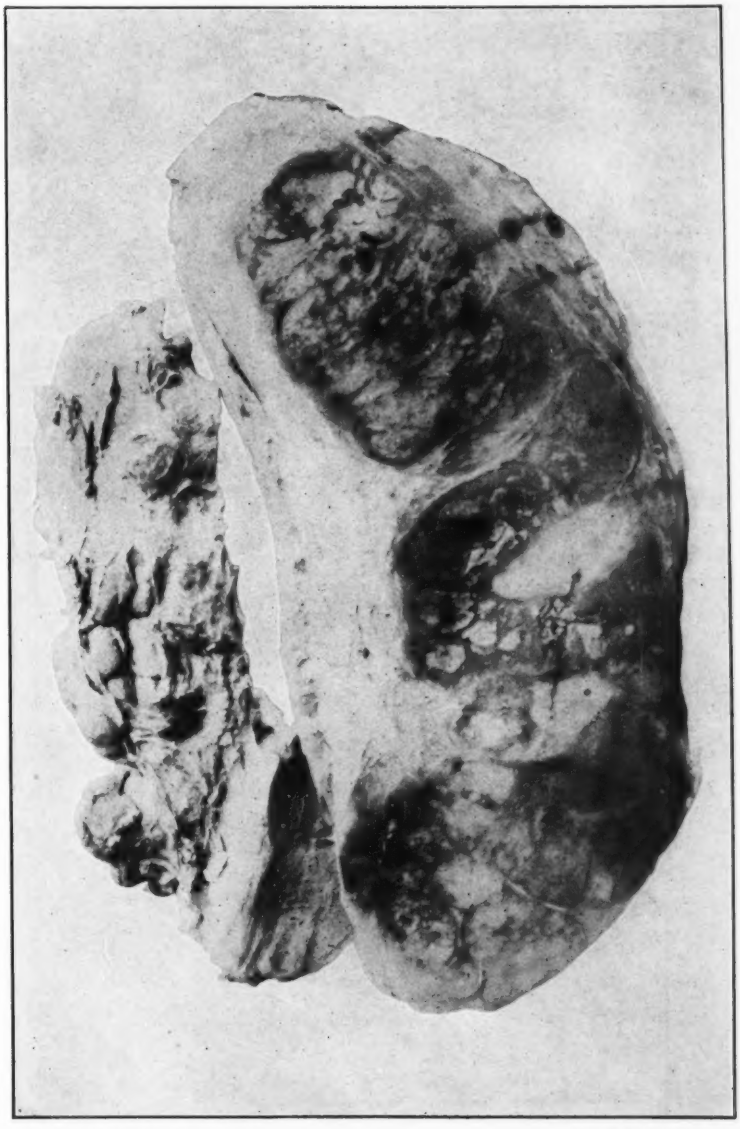
Bact. abortus infection may involve organs of the generative apparatus of bulls, producing chronic inflammatory changes.

Of the generative organs, the seminal vesicles appear to furnish the most favorable site for the lodgment and propagation of abortion infection.

The presence of *Bact. abortus* infection in bulls appears to be more strongly indicated by relatively marked than by slight reactions to the agglutination test for this disease.

PLATE 22

Photograph of normal and diseased seminal vesicles of bull 98, showing the marked increase in size and the gross pathological changes of one of the organs.



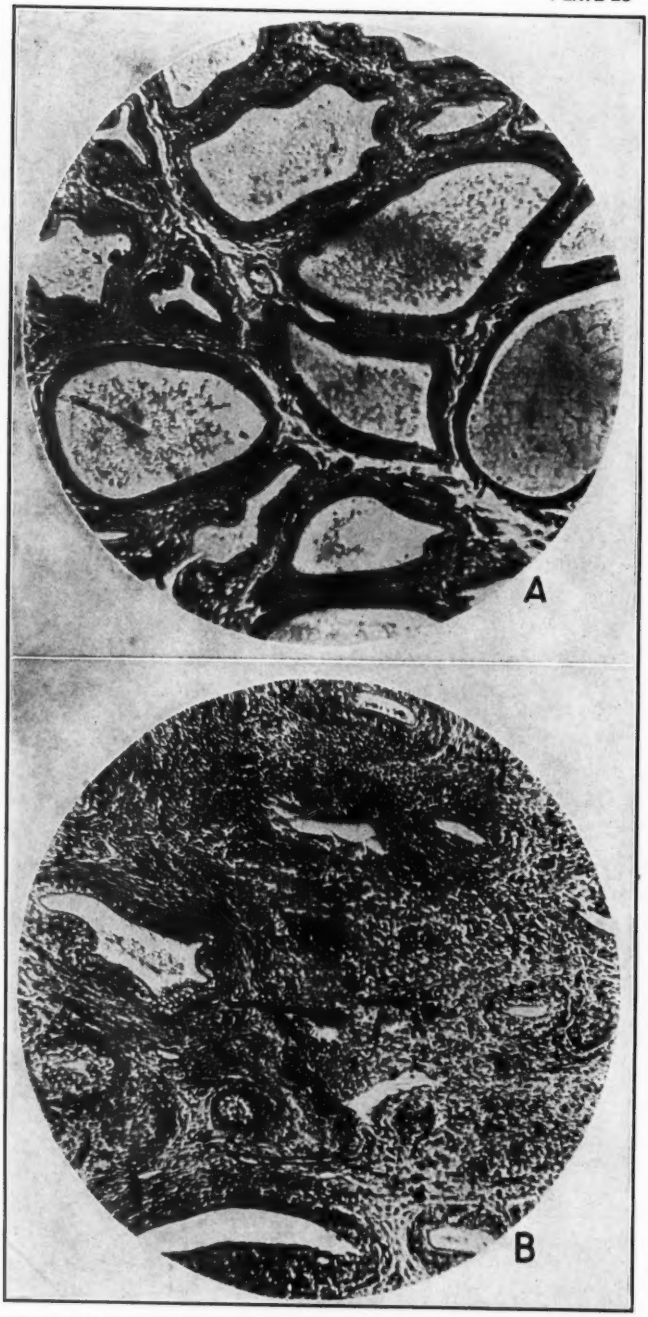

A
B

PLATE 23

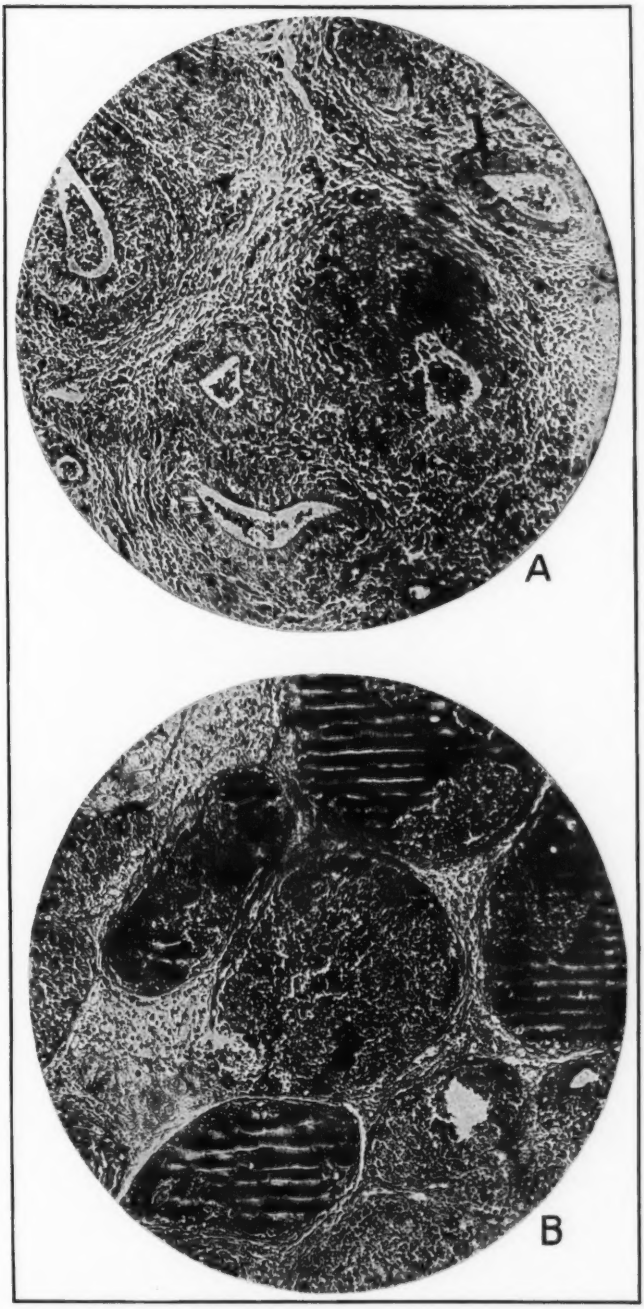
A.—Photomicrograph of a section from a normal seminal vesicle of bull. × 92.

B.—Photomicrograph of section from seminal vesicle of bull 409, showing inflammatory changes. × 92.

PLATE 24

A.—Photomicrograph of section from seminal vesicle of bull 98, showing tissue proliferation and exfoliation of epithelium lining acini. × 92.

B.—Photomicrograph of section from seminal vesicle of bull 98, showing advanced pathological changes with cell degeneration and necrosis. × 92.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